STUDY OF SILVER STAINING OF NUCLEOLAR ORGANISER REGIONS IN MALIGNANCIES OF UTERINE CERVIX

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M.D. DEGREE

In

PATHOLOGY – BRANCH III

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DECLARATION

I hereby declare that the dissertation entitled **STUDY OF SILVER STAINING OF NUCLEOLAR ORGANISER REGIONS IN MALIGNANCIES OF UTERINE CERVIX** was done by me in the Department of Pathology at Coimbatore medical college, Coimbatore during the period from August 2011 to July 2012 under the guidance and supervision of **Dr.C.LALITHA, M.D.,** Additional Professor, Department of Pathology, Coimbatore Medical College, Coimbatore.

This dissertation is submitted to the Tamilnadu Dr.M.G.R.Medical University, Chennai towards the partial fulfillment of the requirement for the award of M.D., Degree in Pathology.

I have not submitted this dissertation on any previous occasion to any university for the award of any degree.

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This is to certify that dissertation entitled **STUDY OF SILVER STAINING OF NUCLEOLAR ORGANISER REGIONS IN MALIGNANCIES OF UTERINE CERVIX** is a record of bonafide work done by **Dr.C.Narmadha,** Post graduate student in the Department of Pathology,Coimbatore Medical College and Hospital, Coimbatore under the supervision of **Dr.M.MURTHY, M.D.,** Professor and Head, Department of Pathology,Coimbatore medical college and Hospital, Coimbatore and under the guidance of **Dr.C.LALITHA, M.D.,**Additional Professor,Department of Pathology,Coimbatore medical college and Hospital ,Coimbatore in partial fulfillment of the regulations of the Tamilnadu Dr.M.G.R.Medical University, Chennai towards the award of M.D.Degree (Branch III) in Pathology.

Guide

Dr.C.LALITHA,M.D.,
Additional professor,
Department of Pathology,
Coimbatore medical college

Dr.R.VIMALA,M.D.,

DEAN
Coimbatore medical college

Dr.M.MURTHY,M.D.
Professor and HOD

Department of Pathology

Coimbatore Medical College

COIMBATORE, TAMILNADU, INDIA - 641 014

(Affiliated to The Tamilnadu Dr. MGR Medical University, Chennai)

ETHICS COMMITTEE

CERTIFICATE

Name of the Candidate : DR. C. NARMADHA

Course : M.D. PATHOLOGY

Period of Study : AUGUST 2011 – JULY 2013

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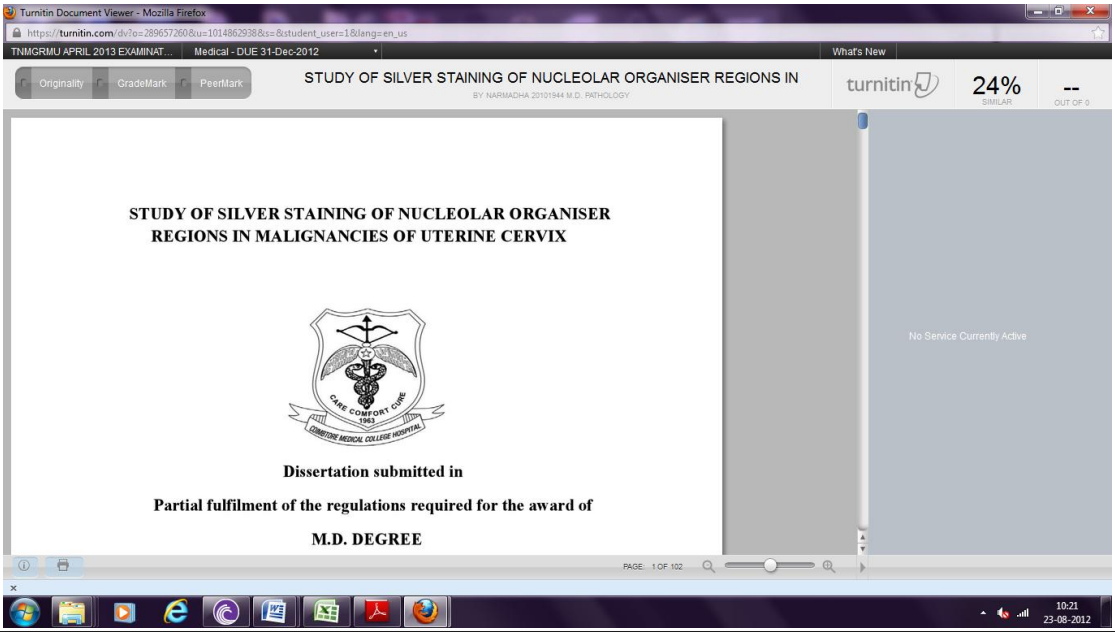
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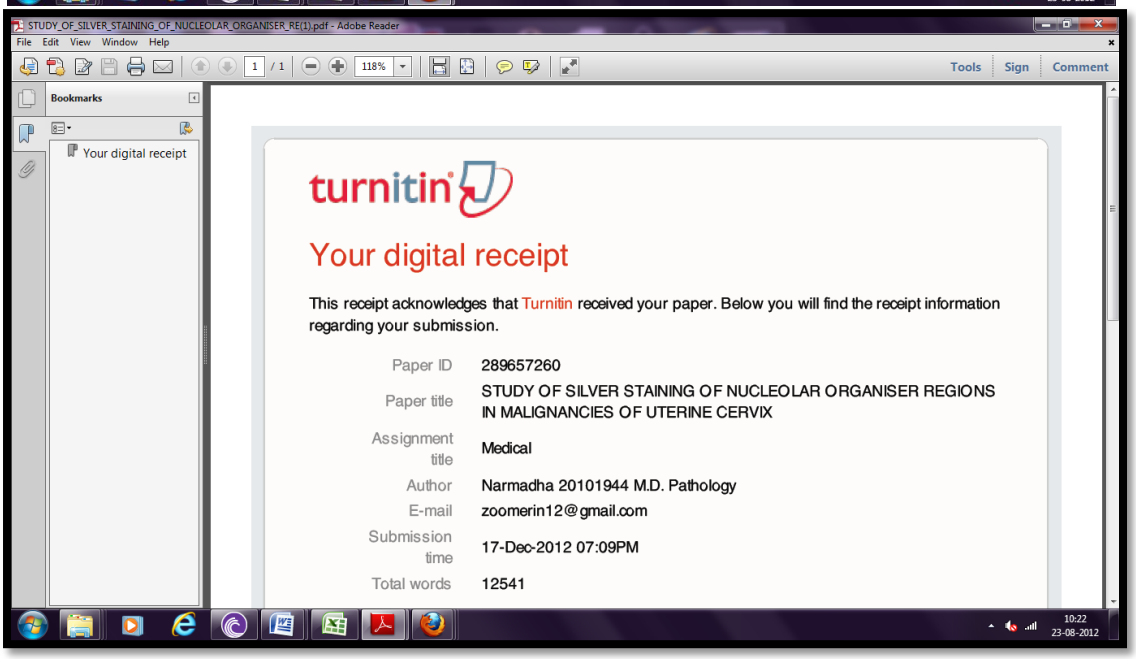
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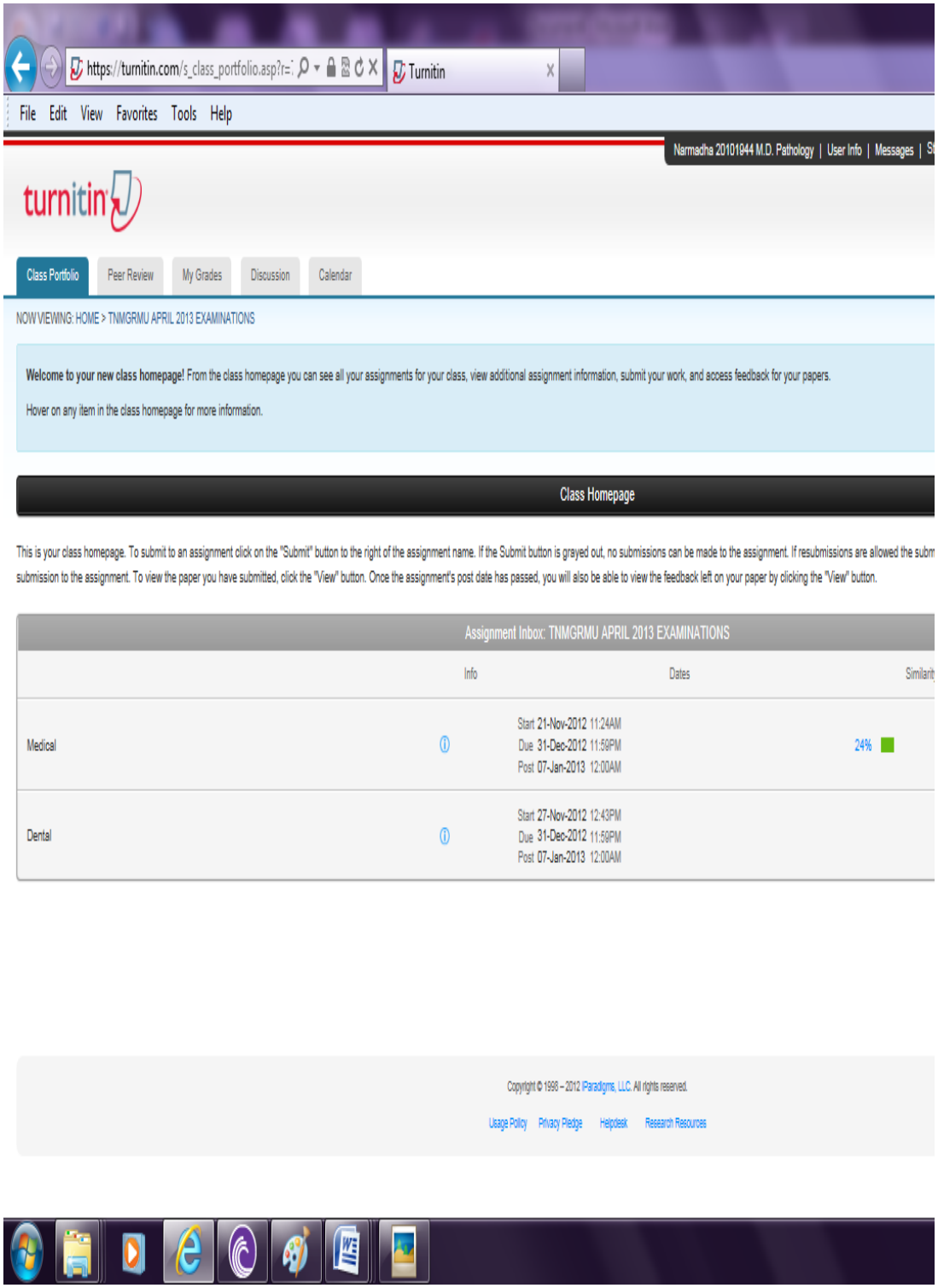
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LIST OF CHARTS

ABBREVIATION

1. **NOR-** Nucleolar Organiser Regions
2. **Ag-** Silver
3. **PAP -** Papanicolaou
4. **PCNA-**Proliferating Cell Nuclear Antigen
5. **IUCD -** Intrauterine contraceptive device
6. **CIN -** Cervical Intraepithelial Neoplasia
7. **CIS -** Carcinoma in situ
8. **WHO -** World health organisation
9. **ISGYP -** International Society Of Gynaecological Pathologist
10. **LSIL -** Low grade Squamous intraepithelial lesion
11. **HSIL -** High grade Squamous intraepithelial lesion
12. **HIV -** Human Immunodeficiency Virus
13. **AIS –** Adenocarcinoma in situ

AIM AND OBJECTIVE OF THE STUDY

1. To statistically analyse the incidence of cancer cervix at Coimbatore Medical College Hospital.
2. To establish the role of AgNORs in differentiating the benign from the pre- malignant and the malignant lesions of the cervix.
3. To establish the grade of malignancy according to the AgNOR count.
4. To evaluate the utility of AgNOR count as a diagnostic and prognostic marker in malignancies of uterine cervix.
5. To plan treatment in cases of carcinoma cervix by using AgNOR count as a prognostic tool.
6. To assess AgNOR count in different types of cervical cancer.

INTRODUCTION

In the female genital tract the cervix is the most common site exposed to both bacterial and viral infection. It is also the target for agents which are carcinogenic leading to invasive cancer.

According to worldwide cancer statistics cervical carcinoma is the second most common cancer in women .Almost fifty percentage of the reported new cases every year have been proved to be fatal. The declining cervical cancer rates are due to accurate histologic interpretation of biopsy specimens by the pathologist and effective Papanicolaou (Pap) smear screening programs [1].

Routine cytological Papanicolaou (Pap) smear screening, early diagnosis and curative therapy has reduced the mortality rate of cervical cancer. Pre-invasive lesions which can progress to invasive cancer have been detected and treated earlier by effective screening programs.

All features which are of diagnostic and prognostic significance are not revealed by routine histopathological techniques. This was due to the difficulties in differentiating the malignant aberrations from the benign ones microscopically.

Therefore, it was essential to develop adjunct procedures which can diagnose malignancy with accuracy and at the earliest.

Studies have showed that there is a definite correlation between nucleolar function, size and the cell doubling time in human cancer cell lines. This concept [2] has stimulated a revolution of the significance of the nucleus in tumour pathology.

Cellular proliferative activity can be assessed by a variety of tests. The method of counting mitosis can detect only major differences in the proliferation of tumour tissue .The procedure of counting mitotic activity is time consuming and also technically difficult.

The disadvantage of flow cytometry is that the tissue is destroyed by the process and it is difficult to evaluate the normal cell population admixed with the cancer cells. This method calculates the percentage of dividing cells by S-Phase fraction.

Markers of cell proliferation like Ki-67, DNA polymerase, PCNA (proliferating cell nuclear antigen) can be demonstrated using immunohistochemical methods. But the procedure is expensive and complicated.

Staining of the nucleolar organising regions by silver compound (AgNOR) has gained popularity for its simplicity and easy procedure. It is of low cost and has good correlation with other proliferative markers.

In proliferating cells, concurrent to the increased synthesis of AgNOR proteins, there is progressive dispersal of ribosomal chromatin, whereas in resting cells ribosomal sequences are located in highly compact structured chromatin.

The frequency of AgNOR count within nucleus are significantly higher in malignant cells than in normal, reactive or benign cell [3,4].

This is a prospective study to see if quantification of AgNOR could help in distinguishing between inflammatory, dysplastic and malignant lesions of the cervix.

REVIEW OF LITERATURE

ANATOMY

The cervix is located in the lower part of the uterus. It connects the uterine cavity to the vagina and a narrow cervical canal passes through it. Ectocervix is the outer surface of the vaginal portion of cervix and endocervix is related to the endocervical canal .The external os is the opening of the endocervical canal into the vagina. Internal os, is the imprecise upper limit of the endocervical canal .[5]

HISTOLOGY :-

Histologically cervical canal is lined by mucus secreting columnar epithelium .It also lines the endocervical glands. In the portio vaginalis the columnar epithelium changes abruptly to non –keratinized squamous epithelium. The connective tissue in the lamina propria of cervix is more fibrous than in the uterus. Blood vessels, smooth muscles, elastic tissue, nerves and occasional lymphatic nodules may be seen.

Smooth muscle fibres are located mainly in the endocervix. It is usually absent in the vaginal portion of cervix.

Sphinctric action at the isthmus is provided by the smooth muscles arranged concentrically which constitutes about 50-60% of the supporting connective tissue.

Most of the exocervix is covered by non-keratinizing squamous epithelium that in child bearing age is composed of three layers basal cell, mid zone and superficial. The real stem cells of the cervical squamous mucosa are located in the parabasal layer according to some authors. A layer of mucus- secreting columnar cells lines the glandular mucosa of the endocervix. This cell which rest on a inconspicuous subcolumnar " reserve" cell located at are near the squamous columnar junction, are primarily involved in the process of squamous metaplasia , Cervical Intraepithelial Neoplasia & carcinoma.

INFLAMMATORY LESIONS OF CERVIX

INFECTIOUS CERVITIS:

The organism most commonly causing active inflammation of the cervix includes bacteria, virus, fungi, protozoa and parasites. Infections frequently seen are due to Candida albicans, Trichomonas vaginalis, Gardenrella vaginalis, Human papilloma virus and Herpes simplex virus.

The squamous epithelium of the cervix is a site of predeliction for the infectious viral agents causing changes in the morphology of the cells which are characteristic.

Causes of non-specific cervicitis not of infectious etiology may be chemical or mechanical in nature. They include tampons, diaphragm, pessaries and IUCD which are foreign bodies causing local trauma. Round cells which includes lymphocytes, plasma cells and histiocytes predominates the inflammatory infiltrate in chronic cervicitis.

The morphological changes seen in association with inflammatory lesions of cervix often cause serious problems in distinguishing from genuine atypia .But the changes in cervicitis are usually mild without reduction in cytoplasm or increase in mitotic activity.

REGENERATING / HEALING EPITHELIUM

Reactive changes can occur in the epithelium due to erosion of cervix by chronic persistent infection .It is also seen in epithelial injury caused by biopsy or conization procedures. The morphological changes are characterized by epithelial disorganization and nuclear atypia seen in squamous and endocervical epithelium.

Histologically and cytologically these reactive changes often mimics Intra Epithelial Neoplasia. In reactive atypia the nuclei are uniform in shape and size, well defined cytoplasmic membrane, clumped chromatin and normal mitotic figures.

ATROPHY:

Hormone deficiency in peri-menapausal, post-menapausal or pre-pubertal states causes alteration in maturation of the squamous epithelium. The changes include uniform basal and parabasal cells with an increased nuclear cytoplasmic ratio, dense chromatin, pleomorphism and rare mitosis.

Epithelial changes seen in the atropic mucosa are sometimes difficult to differentiate from squamous Intraepithelial Neoplasia.

ATYPIA RELATED TO THERAPY:-

Therapeutic level of radiation used in the treatment of cervix can cause morphological changes in both squamous and glandular epithelium.These changes can be acute or chronic.

Radiation effects are variable degrees of epithelial atypia and enlarged nuclei but mitotic figures are absent. Stromal changes include presence of fibrosis, atypical fibroblast and multinucleated cells.

MALIGNANT LESIONS OF CERVIX

PRECANCEROUS DISEASE

Squamous Intra epithelial Neoplasia

The zone of epithelial transformation is the site for all squamous alteration to occur referred to as CIN (Cervical Intra Epithelial Neoplasia)

The region between the original and functional squamo columnar junction is the zone of transformation in which active metaplasia of squamous epithelium occurs. It is the remodeled area of ectropion .Since it is very much dynamic, it is susceptible to changing hormonal and environmental influences.

This area is the one most susceptible to Human PapillomaVirus infection for several reasons, including higher susceptibility of the advancing edge of the immature squamous epithelium to infection.

In 1975, for the first time, the WHO proposed unified terminology to describe and report cervical carcinoma precursor lesions in cervical biopsy specimens. Dysplasia was divided into mild, moderate and severe. It was considered a distinct entity from carcinoma in situ (CIS) in which the entire squamous epithelium was replaced by undifferentiated cells.

In the 1980s, the International Society of Gynaecological Pathologist (ISGYP) introduced nomenclature that replaced the term “dysplasia” with Cervical Intraepithelial Neoplasia (CIN) and eliminated the category of carcinoma in situ. The CIN terminology was divided into three categories, CIN I, CIN II, CIN III, with CIS (Carcinoma in situ) being incorporated into the Cervical Intraepithelial Neoplasia category.

The Bethesda System which has been used for many years in the reporting of cervical cytology is most recently applied for reporting cervical lesions on biopsy specimens.

The Bethesda System is a two-tiered system, which divides dysplasia into LSIL (Low grade squamous intraepithelial lesion) and HSIL (High grade squamous intraepithelial lesion).

Now the single category of High grade squamous intraepithelial lesion incorporates both cervical intraepithelial Neoplasia-II and Cervical intraepithelial Neoplasia-III.

The Bethesda System also places condyloma acuminatum into the LSIL category whereas WHO and CIN terminology kept condyloma acuminatum as a separate category.

The biology of precursor lesions of carcinoma cervix is more closely reflected by the Bethesda System of classification. It also unifies the

nomenclature used for reporting both cervical cytology and biopsy specimens.[8]

PRECURSOR LESIONS OF CERVICAL CANCER

CLASSIFICATION BASED ON DEGREE OF DYSPLASIA	**TERMINOLOGY ACCORDING TO WORLD HEALTH ORGANISATION.**	**BETHESDA SYSTEM**
Mild dysplasia	CIN 1	Low –grade squamous intraepithelial lesion(LSIL)
Moderate dysplasia	CIN 2	High –grade squamous intraepithelial lesion(HSIL)
Severe dyplasia /carcinoma in situ	CIN 3	High –grade squamous intraepithelial lesion(HSIL)

*World Health Organization, Cervical Intraepithelial Neoplasia (CIN)

LOW GRADE SQUAMOUS INTRAEPITHEIAL LESION (LSIL)

Squamous intraepithelial lesions which are low grade typically occurs in women of reproductive age , with the peak incidence in the third decade as most young women are exposed to Human Papilloma Virus sometime after becoming sexually active.

Three morphologic subtypes of Low grade squamous intraepithelial lesions are;

1) Condyloma acuminatum
2) Condyloma -immature
3) Condyloma -Flat

CONDYLOMA ACUMINATUM

The histology shows papillae which are blunt shaped with a blood vessel in its core, acanthosis and superficial koilocytic changes. Underlying cervical stroma shows chronic inflammatory cells.

CONDYLOMA-IMMATURE

The features seen are papillae which are slender lined by squamous epithelium showing little keratinocyte maturation and mild superficial koilocytic atypia.

FLAT CONDYLOMA:

It exhibits morphological features similar to condyloma acuminatum but the pattern of growth is not exophytic here. Upper third of the epithelium shows atypical changes in this lesion.

HIGH GRADE SQUAMOUS INTRAEPITHELIAL LESION (HSIL):-

These occur at a slightly older age group then LSIL, with highest prevalence in women between 35 to 39yrs.The frequency of HSIL has increased in younger women over the past 20 years. The epithelium shows atypical nuclear features involving all the layers with a variable degree of surface maturation.

Cervical intraepithelial Neoplasia-II shows epithelial maturation and koilocytic changes in surface epithelium. In case of severe dysplasia /in situ carcinoma epithelial maturations is minimal or absent.[10].

Cervical intraepithelial Neoplasia-III includes both severe dysplasia and carcinoma in situ.

According to an international committee held first on exfoliative cytology, carcinoma in situ is defined as a preinvasive lesion with a lining surface epithelium showing no differentiation at all levels of epithelium.[7]

A more aggressive approach to the management of these lesions is warranted, because progression to invasive carcinoma occurs in a higher percentage of these lesions [20].

INVASIVE CARCINOMA OF CERVIX:-

The cervix is the commonest site for female genital tract cancer and statistics vary considerably from country to country and from race to race. So in African and Asian women living in poor conditions, the incidence and relative mortality rate of the carcinoma cervix is 4-5 times higher than those seen in developed countries.[6, 11]

Carcinoma cervix is the second most common cancer in women worldwide next to breast cancer incidence. Cancer mortality statistics states that it is the third leading cause of death after lung and breast cancers.

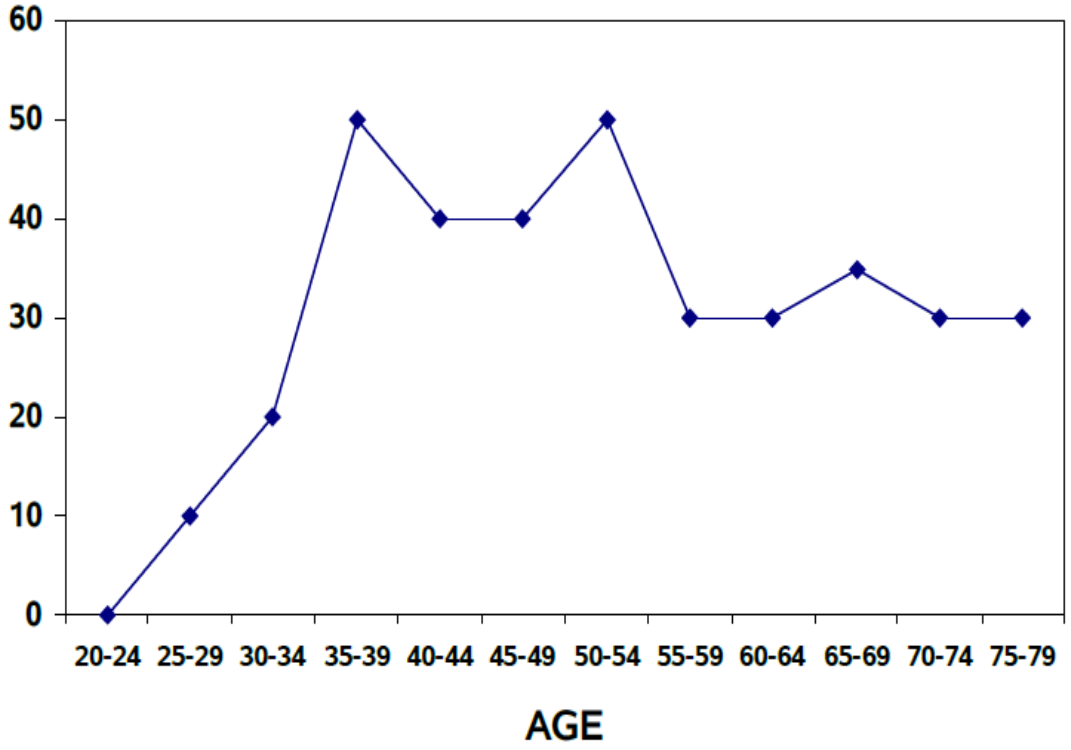
Cancer of cervix is reported at all ages of women. Invasive squamous cell carcinoma is being diagnosed at two peaks one at about 35 years and another around 50-55yrs.

AETIOLOGY

Age

Young sexually active women are at increased risk for Human Papilloma virus infection and preinvasive cervical intra epithelial Neoplasia. This risk drops significantly with increasing age which is associated with increasing risk of cancer. Risk drops further with the approach of

menopause. .



Cervical Intra epithelial Neoplasia (CIN) occurs at a much lower age. One third of the cases being found in women less than 30 years old.[15, 16]

Religion and Race:

The incidence of cervical cancer is less in women of Jews and Muslims due to ritual circumcision done at childhood in male children.

Social and Economic Factors:

Cervical carcinoma is common in people living in low Socio economic status .They are very much more prone due to low standards of penile hygiene, early coitus, frequency of sexual intercourse and promiscuity of both partners.

Coitus:

Incidence of cancer is 4-5 times more common in sexually active women than in sexually inactive women.

Cervical Infection:

Human papilloma virus plays an important role in virtually all cases of which type 16, 18, 31 and 45 accounts for 80% of cervical carcinoma.

There are about 15-20 types of HPV acting as a cofactor in epithelial cancer of cervix. HPV 16 is associated with 50% of cases.[13,17]

Hormonal Factors:

There is some evidence that Estrogen- Progesterone Oral Contraceptive pill usage favours CIN changes and also for Adenocarcinoma.

The other risk factors in epidemiology of cervical cancer include HIV infection, deficiency of vitamins, cigarette smoking and pregnancy at early age.

Predisposing Histological Status:

Certain histological changes in the cervix which are alleged to be "Pre cancerous" or which are sometimes confused with cancer include basal Cell hyperplasia, squamous cell metaplasia and CIN. Of these, only CIN-II and CIN III are likely to significant forerunners of invasive carcinoma.[18, 19]

Histological Classification of Cancer Cervix:

Carcinoma cervix nearly always starts at the squamo-columnar epithelial junction and 80-90% cases are squamous cell in type.

In 5-10% of cases it is entirely columnar cell in pattern (AdenoCarcinoma) and the frequency is increasing especially in young women who are smokers or pill-users. The remainder constitutes the mixed types.

WHO Histological Classification - The Uterine Cervix Tumors

Epithelial Tumors

Squamous tumors and precursors

Squamous cell carcinoma, not otherwise specified

Non- Keratinizing

Keratinizing

Papillary

Basaloid

Warty

Verrucous

Lymphoepithelioma –like

Squamotransitional

Early invasive (micro invasive) squamous cell carcinoma

Squamous intraepithelial Neoplasia

Cervical intraepithelial Neoplasia-3 (CIN3)

Squamous cell carcinoma in situ

Benign squamous cell lesions

Squamous papilloma

Condyloma acuminatum

Fibroepithelial polyp

Glandular tumors and precursors

Adenocarcinoma

Mucinous adenocarcinoma

- Endocervical
- Intestinal
- Signet-ring cell
- Minimal deviation

Endometrioid adenocarcinoma

- Clear cell adenocarcinoma
- Villoglandular

Serous adenocarcinoma

Mesonephric adenocarcinoma

Early invasive adenocarcinoma

Adenocarcinoma in situ

Glandular dysplasia

Benign glandular lesions

- Endocervical polyp
- Mullerian papilloma

Other epithelial tumors

- Adenosquamous carcinoma
- Glassy cell carcinoma variant
- Adenoid cystic carcinoma

Adenoid basal carcinoma

Neuroendocrine tumors

Carcinoid

Atypical carcionoid

Small cell carcinoma

Large cell neuroendocrine carcinoma

Undifferentiated carcinoma.

Mesenchymal tumors and tumor – like conditions

Leiomyosarcoma

Endometrioid stromal sarcoma, low grade

Undifferentiated endocervical sarcoma

Sarcoma botryoides

Leiomyoma

Genital rhabdomyoma

Alveolar soft part sarcoma

Angiosarcoma

Malignant peripheral nerve sheath tumor

Postoperative spindle cell nodule

Mixed epithelial and mesenchymal tumors

Carcinosarcoma (malignant mullerian mixed tumors)

Adenosarcoma

Wilms tumor

Adenofibroma

Adenomyoma

Melanocytic tumors

Malignant melanoma

Blue naevus

Miscellaneous tumors

Tumors of germ cell type

Dermoid cyst

Mature cystic teratoma

Yolk sac tumor

Lymphoid and haematopoetic tumors

Malignant lymphoma (specify type)

Leukaemia (specify type)

Secondary tumors.

Reagan et al (1957) recognized three groups of squamous cell carcinoma[27]

- Non – Keratinizing type –large cell type (moderately differentiated grade)
- Keratinizing type- Large cell type (well differentiated grade)
- Small cell Non – Keratinizing type(poorly differentiated grade)

Other variants of squamous cell carcinoma are

- Papillary squamo transitional carcinoma
- Verrucous carcinoma
- Lympho epithelioma -like
- Basaloid
- Condylomatous (warty)

MICROINVASIVE SQUAMOUS CARCINOMA:-

Mestwerdt was the first to introduce the concept of microinvasive carcinoma in 1847. In the progressive spectrum of squamous intraepithelial Neoplasia[25] it is considered to be a stage which is preclinical. It is a carcinoma that could be diagnosed only by histological examination.

It is defined as a tumour that must be identified only by examination by microscope. Invasion by tumour extends to a maximum depth of 5mm and a maximum width of 7mm.

INVASIVE SQUAMOUS CELL CARCINOMA

Worldwide squamous cell carcinoma of cervix is the second most common cancer in women after breast cancer. Squamous cell carcinoma is the most common cancer in women in developing countries

Although previously accounting for >90% of all cervical cancers, the overall frequency has decreased due to the implantation and success of national cervical smear screening programs. This has also resulted in the detection of otherwise asymptomatic small, early invasive lesions.

Squamous cell carcinoma of cervix has three variants[27]

- Non –keratinising carcinoma which account for two thirds of cases show cytoplasmic keratinization of individual cells and intercellular bridges, but keratin pearls and nests of tumour cells with central keratin are not present.
- Keratinizing carcinoma which account for one –sixth of cases contain keratin pearls and nests of tumour cells with central keratin ; cytoplasmic keratinization and keratohyaline granules are also present and intercellular bridges can be identified.

- Small cell non -keratinizing carcinoma are characterized by sheets of smaller non-keratinized cells, with high nuclear cytoplasmic ratio.

Epidermoid (squamous cell) carcinoma comprises the majority of cervical carcinoma about 70%. The most common pattern is non-keratinising large cell type. It has better prognosis.

Well –differentiated keratinizing epidermoid carcinoma constitutes about 25% of the cases .Undifferentiated carcinoma-small cell is less common and has a poor prognosis.

Modification of Broder's method[28] –based on the extent of differentiation is

- Well differentiated- Grade-1
- Moderately differentiated- Grade-2
- Poorly differentiated -Grade-3

Most are grade-2, then 3 and then 1.

Basaloid carcinoma have a basaloid appearance with nests of tumour cells having less abundant eosinophilic cytoplasm and peripheral pallisading of nuclei.

Verrucous carcinoma- extremely rare subtype is characteristically exophytic with little to no cytologic atypia.

Warty carcinoma is exophytic with surface koilocytic changes.

Papillary carcinoma is characterized by papillary growth pattern and divided into –

1) Papillary undifferentiated carcinoma

2) Papillary transitional cell carcinoma

3) Papillary squamo- transitional carcinoma

Lymphoepithelial like:[9]-

It is characterized by ill defined islands of undifferentiated cells associated with marked lymphocytic background within the stroma. The tumour cells have a syncytial growth, poorly defined cell borders and moderate pale eosinophilic cytoplasm.

Adenocarcinoma:

Primary adenocarcinoma makes up 15% of all carcinoma cervix. It's relative incidence is on the rise , particularly young women. An

association has been found between long term use of OCP and the development of endocervical Neoplasia in young patients.[5]

Most common pattern is that of a well differentiated glandular pattern with mucin secretion.

Morphological variants

- Endometriod adenocarcinoma
- Papillary serous carcinoma
- Adenosquamous (mixed carcinoma)
- Adenoid cystic carcinoma
- Adenoid basal carcinoma
- Clear cell carcinoma

Endometriod adenocarcinoma resembles its endometrial and ovarian counterparts. There is an increasing incidence in this type carcinoma

Adenoma malignum is minimal deviation adenocarcinoma.

Glucksmann and Cherry originally emphasized the importance of Adenosquamous carcinoma and used the term Mixed carcinoma.[7] Mixed carcinoma combines pattern of adenocarcinoma with well defined squamous component.

A distinct type of poorly differentiated adenosquamous carcinoma referred as glassy cell carcinoma occurs at an age group younger than cervical neoplasm. It has often been associated with pregnancy.

Clear cell carcinoma (mesonephric carcinoma) is composed of glands in tubule-cystic, papillary pattern with a single lining of cells having large amount of clear cytoplasm .The cells may show enlarged and pleomorphic nuclei protruding into the lumen giving a 'Hob nail'appearance.

Although it can occur in all age groups it is common in young females and the prognosis is relatively good.

Mixed epithelial / Mesenchymal neoplasms:

It includes:

1. Endocervical polyp
2. Adenomyoma
3. Carcinosarcoma
4. Cervical Adenosarcoma

Adenosarcoma:

It is a biphasic tumour composed of a malignant stromal component and a benign epithelial component. Primary cervical adenosarcoma is extremely rare accounting for a minority (2%) of those that occur in the genital tract.

Melanocytic Tumours

1. Blue nevus

2. Malignant melanoma

Only 30 cases of malignant melanoma have been reported in the literature. It is a neoplasm which is very rare. The mean age of patient is in the sixth decade and the prognosis is poor.

Neuroendocrine tumours

The group of neuroendocrine tumours includes

- Carcinoid
- Atypical carcinoid
- Large cell neuroendocrine carcinoma
- Small cell carcinoma

Carcinoid

Benign carcinoids have characteristic organoid appearance as seen in other sites. The differential diagnosis between typical and atypical carcinoids is based on the atypia of nucleus and mitotic activity.

Atypical carcinoid

Increased mitotic count (about 5-10 per high power field), areas of necrosis and cytologic atypia are features of atypical carcinoid.

Small cell carcinoma

This type of carcinoma account for 1-6% of cervical carcinoma. Squamous or glandular differentiation may be present.

Large cell neuroendocrine carcinoma

It has features of high grade neoplastic cells with trabecular or insular pattern at least in a focal area. The tumour cells have vesicular chromatin, abundant cytoplasm, prominent nucleoli and high mitotic index (greater than 10 mitosis per 10 HPF).

Metastasis from neuroendocrine carcinoma, sarcoma-undifferentiated, neuroendocrine differentiation in adenocarcinoma are the differential diagnosis. Markers like neuron specific enolase,

chromogranin and synaptophysin are used in immunohistochemistry to show neuroendocrine differentiation.

The misery of women due to carcinoma cervix, which causes increased mortality in women is a scourge of humanity in a country developing like ours.

Best approach to achieve control over cancer cervix is early diagnosis and management of the intraepithelial lesions of squamous epithelium.

Cytological screening by Pap smear has brought down the incidence of cervical cancer in developed countries [35].Unfortunately cytology fails to identify high risk low grade SIL (LSIL) and high grade SIL (HSIL) which would progress to invasive cancers. Such information can be provided by a molecular tumor marker[36]. One such molecular tumor marker is AgNOR which stands for silver stained (Ag) nucleolar organizer regions (NORs).

NUCLEOLAR ORGANIZER region (NORS)

Ribosomal RNA is encoded by the DNA loops present in the nucleoli of all cells. These are called the Nucleolar organizer region (NORS).

The acrocentric chromosomes 13, 14, 15, 21and 22 showing secondary metaphase constrictions in their short arms are referred to as NORS.

The technique of silver impregnation can be used to detect these regions which are argyrophilic [21].

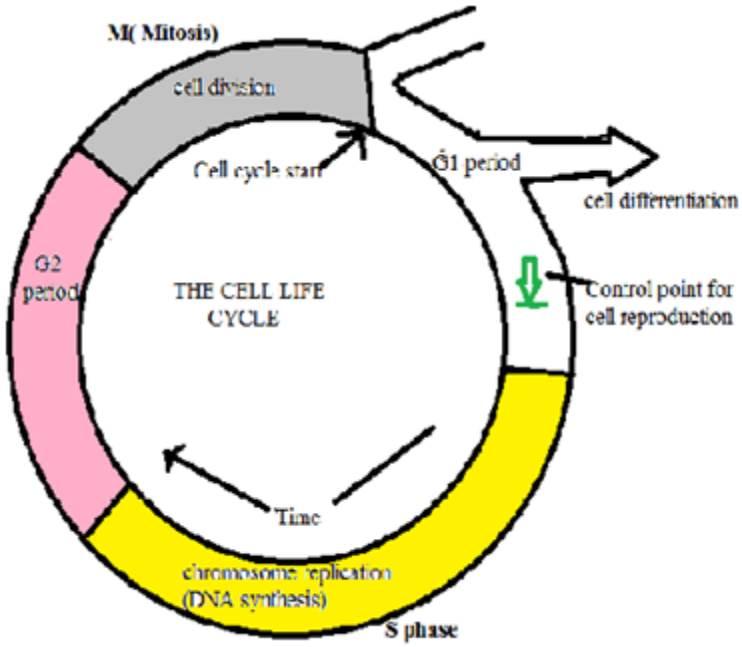
Silver stainability or NOR is due to the presence of a peculiar set of acidic proteins , which are highly argyrophilic.[23] .Binding of silver and protein occur in carboxyl and sulphydryl groups by colloidal precipitation of ionic silver. The silver solution is reduced by the carboxyl groups forming micronuclei of silver.

Deposition of large aggregates of silver occurs at the disulphide and sulphydryl groups.

These are visible by light microscope as black intra nuclear granules.

The number of AGNOR in a cell nucleus reliably reflects the cell kinetics and proliferative activity.[22]

Cell kinetics plays an important role in tumour behavior and proliferation. Rates of tumour proliferation can be assessed to determine the behavior of particular tumour.[14]



The cell cycle can be divided into four phases based on the nuclear chromatin activity .They are S,G1 G2 & G0 .The DNA content at the end of "S" phase is an indicator of proliferative activity and AGNOR detects the DNA content at this stage.

STRUCTURE AND FUNCTION OF INTERPHASE NOR:-

Ribosomal genes essential for biogenesis are present in the nucleolus which is a well defined functional and structural unit of the cell.

Three main components of the mammalian cell nucleoli apart from intranuclear chromatin are;

1) The fibrillar center, appears as rounded structures of different size and is composed of very thin loosely, inter oven fibrils.
2) Densely packed fibrils present at the periphery of the fibrillar center constitutes the dense fibrillar component.
3) Fibrillar component is surrounded by granules which is the granular component.

Inter phase counterparts of metaphase NOR are the fibrillar centers and the closely associated dense fibrillar component [24] . Structural-functional units for rRNA transcription are represented by the interphase NOR.

Ultra structural and immuno-cytochemical investigations in the interphase NOR revealed the presence two main enzymes RNA polymerase I and topoisomerase I controlling transcription.

The two main AgNOR proteins called as Protein B23 and Protein C23 plays a vital role in the control of transcription and processing of rRNA. [37]

Interphase NOR and Nucleolar Pathology:

Simple staining methods using the principle of silver impregnation identifies the proteins which are argyrophilic. These are AgNOR which stains as black dots in the nucleus.

AgNOR content is expressed as mean AgNOR count for 50-100 cells. After DNA synthesis, in the G2 phase of the cycle a diploid nucleus might contain 20 AgNOR. In practice the AgNOR are so closely packed and it is difficult to distinguish one AgNOR from another. The argyrophilia of the NOR associated protein (NORAPs) acts as marker of ribosomal DNA and reflects its transcriptional phase. The number of visible AgNOR therefore indicates the current phase of transcription[26]

Ribosomal sequences of the chromatin is highly compact in resting cells whereas in proliferating cells progressive dispersal of ribosomal chromatin occurs due to increased synthesis of AgNOR.

Clinical management of cancer is based on the histopathological typing, grading and staging of tumours. But clinical outcome in many cases has no significant correlation with the histopathological diagnosis.

All possible markers of prognostic importance may not revealed by histopathological assessment. These problems have led to the

development of newer techniques to be used as an adjunct to routine methods available.

To improve the accuracy and reproduce the features of prognostic significance[29] a new technique was introduced based on nuclear studies. This assesses tumour tissue by silver staining of the nucleolar organising regions (AgNOR).

There is significant high frequency of AgNOR dots within nucleus of malignant cells than in normal, reactive or benign neoplastic cell. Good correlation with other markers of proliferation, reasonable cost and simple procedure has made this technique popular.[31]

Their potential value in diagnostic histopathology was they can be easily demonstrated in routinely processed tissue sections.

The cell population in tissue sections shows an apparent increase in the mean AgNOR count if:

1. Cell proliferation was so active and nucleolar dissociation was present in many cells, when the AgNOR were seen throughout the nucleus.

2. There was a defect of the nuclear association, resulted in AgNOR dispersion.

3. There was an increase AgNOR following increased cell ploidy.

4.Prominence of inconspicuous AgNOR as a result of increased transcriptional activity.

In a normal cell 20 black dots of AgNORs should be seen (2 per arm of chromosome i.e. (2x 10 = 20) but only one or two dots are seen as the dots are tightly packed [34]. As we move from normal cells towards the dysplastic cells and malignant cells, the amount of DNA increases, and the number of AgNOR dots (AgNOR count) also increases.

INTERPHASE AgNOR AND CANCER PROGNOSIS:-

Until recently, at light microscopic level a precise evaluation of nucleolar morphology, and its change during cell activation or transformation was impossible to be performed in routine cytopathology and histology.

Small nucleoli are usually undetected and large nucleoli, independent of their size and shape are generally defined as “Prominent”.

In 1986, Ploton and co-worker applying the formic acid silver nitrate staining method for AgNOR proteins at the light microscopy level, succeeded in a very precise visualization of interphase NOR.

Evaluation of interphase NOR which appear as well defined black dots is done by light microscopy and each dot correspond to one interphase NOR as visualized by the electron microscope.[24]

There is a definite relationship between the proliferative activity of cells and interphase AgNOR. Hence quantification of interphase AgNOR's can be considered as a parameter of cell kinetics. The shorter the doubling time of cell the greater the amount of interphase AgNOR .[38]

Ploton and co-workers have demonstrated that there is greater quantity of interphase AgNOR in malignant cells than the benign or normal cells and therefore evaluation of interphase AgNOR distribution may be useful for the diagnosis of malignancy[24]

The cell kinetic parameters may help clinical oncologists and pathologist to define the biological behaviors of cancer lesions, indications about specific treatments of cancer and valuable information of prognostic significance.

Previously stained cytology slides can be reused for silver staining providing an excellent guide to the diagnosis especially in doubtful cases

and when unstained slides are not available. This is one of the great advantages of this silver staining technique.

The major disadvantages are:

1. Observer error is the major cause of inaccuracy and inconsistency
2. The counting procedures adopted are usually manual and hence long and tedious.
3. Overlap and coalescence may result in misjudged counts [26].

There has been a growing interest in the study of DNA and proliferation markers. One of the most recent studies is on Nucleolar Organizer silver reduction technic method.

D C Rowlands[37] (1988) was the first to study the number of nucleolar organising regions (NORs) among different grades of cervical intraepithelial lesions (CIN).

A small but significant increase in the CIN-III group and no significant difference in the AgNORs in the normal biopsy specimens of squamous epithelium and those showing CIN-I and CIN-II were observed.

Mark Egan et al [36] (1990) studied the relationship between intraepithelial neoplasia of the cervix, and the size and number of nucleolar organizer regions. They found that mean numbers of AgNORs

identified steadily increased, whereas the mean sizes of AgNORs decreased from CIN I to CIN III.

CIN III could be distinguished from CIN I and CIN II on the basis of AgNOR sizes, and an inverse relationship between AgNOR numbers and sizes was established.

Darne et al[43] *(*1990) in their study on AgNORs in normal endocervix, adenocarcinoma and in AIS observed a significant overlap between cases of invasive adenocarcinoma and Adenocarcinoma in situ (AIS).

There was no overlap between the groups of normal endocervical cells and invasive adenocarcinoma.

This suggests that AIS is a precursor lesion of invasive adenocarcinoma. But histological subtypes of adenocarcinoma could not be differentiated using assessment of AgNORs.

Yasuhiro Yokoyama et al[12] (1990) in their study on Nucleolar organizer regions (NORs) in 70 cases of precancerous and cancerous lesions of the uterine cervix found that the mean number of AgNOR dots in the cases of mild dysplasia that progressed to CIS was 2.7. This was significantly higher than that in the cases that regressed. No correlation was noted between CIS and invasive carcinoma.

Lakshmi et al[44] (1993) observed a highly significant positive correlation between AgNOR counts and tumour progression in their AgNOR analysis done in CIN lesions and invasive squamous cell carcinomas. Maximum AgNOR counts were seen in invasive carcinomas.

Kaushik R et al [39] (1995)studied hundred cases of cervical lesions including normal, chronic cervicitis, CIN (I, II, III) and carcinoma to assess the utility of AgNOR counts in differentiating cervical lesions. Statistical counts of AgNORs were done. The mean AgNOR counts in cervical epithelium showed a progressive and statistically significant increase from normal to chronic cervicitis to CIN I, II and III ($P<0.001$). Scores in carcinoma also exceeded that of CIN ($P<0.05$).

D Prathiba et al [24] (1995) analysed AgNOR count in premalignant and malignant lesions of cervix and found statistically significant difference in these lesions. They also found that marked disaggregation of AgNORs occurs in small cell carcinoma of cervix indicating very high proliferative activity and poor prognosis.

Jyothima Agarwal et al[46] (1997) counted AgNORs in biopsies from various lesions of cervix. The mean of AgNORs per nucleus was significantly higher in CIN (4.05±0.04) and malignancy (5.50±0.65) as compared to squamous metaplasia (1.74±0.32) and chronic cervicitis

(1.54±0.42). Adenocarcinoma had higher counts compared to other carcinoma.

Pillai et al [45] (1998) in their study observed that increased proliferative activity may be a positive indicator of prognosis i.e. tumours with high AgNOR counts and Ki67 index responded better to radiotherapy. Pre-treatment biopsies from 152 squamous cell carcinoma patients were evaluated for Ki67 expression and AgNOR counts to relate the disease outcome after radiotherapy.

Murty et al[59] in their study found that not only the count of NORs increases in carcinoma cervix but also they become more prominent and larger in size appearing as giant size.

Kari J.Syranen et al [41] (2001) were the first to analyse the prognostic significance of AgNOR in 85 cases of squamous cell cancer of cervix with known HPV status.[42] Significant correlations of AgNOR counting and quantitation with tumour proliferation and progression in cervical squamous cell carcinoma was found.

Ritu Singhal et al[50] **(**2003) found that in squamous cell carcinoma cervix the AgNOR counts were higher when compared to glandular carcinoma of cervix.

Pahuja S et al[51] (2003) studied the proliferative potential of squamous intraepithelial lesions in fifty biopsy sections of various grades of preinvasive and invasive squamous epithelial lesions of cervix by AgNOR staining. AgNOR counts showed progressive rise in their mean value with increasing grade of lesion. Amongst invasive malignancies, highest mean of AgNORs were observed in poorly differentiated squamous cell carcinoma of cervix.

They concluded that proliferation potential of squamous intraepithelial lesions of cervix is the main factor affecting the biological aggressiveness of the lesion.

Kafil Akhtar et al[47] (2005) conducted a study on 50 histologically proven, previously untreated cases of different grades of squamous cell carcinoma of the cervix. The patients were subjected to Cobalt-60 radiotherapy and pap smears obtained 4 and 8 weeks after therapy were assessed for AgNOR score.

AgNORs in carcinoma of the cervix prior to radiotherapy were large and variable in size and shape and after radiotherapy showed multiple AgNOR dots which were fewer in number and less coarse as compared to the pre-radiation group. In patients who showed persistence of malignancy, the AgNOR dots were found to be coarse and present in large clumps.

Singh Uma et al [48] *(2006)* observed in their AgNOR study of 43 histologically proven cases of CIN that mean AgNOR count per cell was more in CIN II than that in CIN I and more in CIN III than that in CIN II. They concluded that AgNOR count can be used as an adjunct to histology for diagnosing CIN especially in doubtful cases.

Vijaya V Mysorekar et al[49] (2008) reviewed two hundred specimens of benign and malignant squamous epithelial lesions of the cervix to study the utility of proliferative and apoptotic indices .They found that both indices combined are useful to differentiate between benign, preinvasive and invasive squamous epithelial lesions of the cervix.

They concluded that the prognosis and plan of management in cases of cervical lesions can be determined by mitotic count, AgNOR count and apoptotic count which are the simplest techniques that can be employed in any laboratory.

Luz del Carmen Alarcón-Romero et al[66] studied about the AgNORs polymorphisms in squamous intraepithelial lesions (SIL) and squamous cell carcinoma (SCC) with HPV infection. They found that according to the grade of histological lesions AgNORs polymorphism rises progressively. It is a useful prognostic indicator.

KC Shiva Raj et al[40] (2012) studied the role of AgNOR in differentiating non-neoplastic and precursor lesions from neoplastic lesions in both cervical smears and histology by conducting a prospective study.

They found that the number and shape of AgNOR dots change from benign to precancerous to malignant tumors and AgNOR Pattern Assessment is useful in differentiating benign to intraepithelial lesions to carcinoma cases.

MATERIAL AND METHODS

Total 62 female patients were studied, out of them 57 were of cervical pathology and 5 were used as normal controls. Detailed histological examination in hematoxylin and eosin was done along with the AgNOR studies. The AgNOR score was then evaluated in relation to the histological diagnosis.

This is a prospective study that included biopsy specimens of benign, pre-malignant and malignant squamous epithelial lesions of cervix.

INCLUSION CRITERIA

These included cervical biopsies done for diagnostic evaluation, as well as surgical specimens resected as a therapeutic measure.

EXCLUSION CRITERIA

* Biopsies showing extensive ulceration of the cervical squamous epithelium were excluded from the study.
* Sections not showing adequate epithelial cells for counting were also excluded.

* Tumours with extensive necrosis and scanty viable tissue were also excluded.

Single step AgNOR staining technique[52] was employed on 3microns thick paraffin sections for demonstration of AgNORs.

Silver nitrate method for AgNOR protein sites (after Ploton et al 1986)

Sections:

Formalin –fixed, 2-3-µm paraffin sections.

Solutions:

50% silver nitrate solution

Silver nitrate -50g

Distilled water - 100ml

Gelatin solution

Gelatin -2g

Formic acid -1ml

Distilled water -100ml

WORKING SOLUTION

Silver nitrate solution 2 parts by volume

Gelatin solution 1 part by volume

Mix in above proportion immediately before use, the volume of working solution used depends on the number of slides to be stained.

STAINING METHOD

1. Dewax sections in xylene, hydrate through ethanol to water.
2. Rinse sections in distilled water.
3. Incubate in freshly prepared working solution for 30-40 min at room temperature.
4. Wash in distilled water for 1min.
5. Dehydrate , clear, and mount in non-aqueous mounting medium.

RESULTS

AgNOR sites -intranuclear black dots

Background -yellow

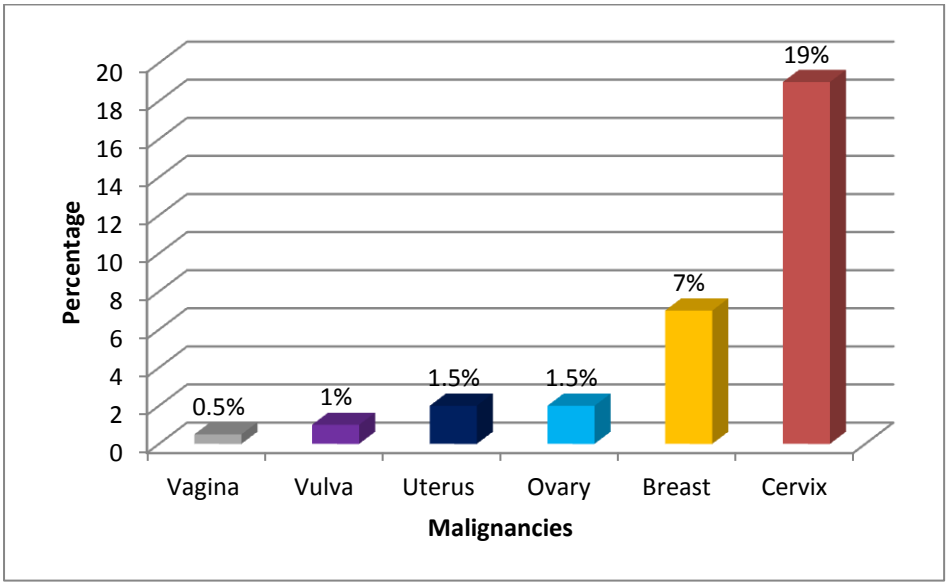
OBSERVATION AND RESULTS

Of 2136 gynecological specimens received at pathology department Coimbatore Medical College Hospital during the period August 2011-July 2012, cervical intraepithelial Neoplasia was reported in 12cases and malignant lesions of cervix in 229 cases.

This prospective study included routinely processed biopsies from 62 patients with various lesions of the cervix diagnosed histologically during the period August 2011-July 2012.

According to the cancer statistics 2011at Pathology department Coimbatore Medical College Hospital, the incidence of malignancies in the female reproductive system is as follows Cervix 19%,Breast 7%,Uterus 1.5%, Vulva 1% and Vagina 0.5% .

The incidence of malignancies in the female reproductive system in given in the following chart.



AGE INCIDENCE

The patients initially diagnosed as Cervical Intra epithelial Neoplasia and Cervical cancer were divided into 7 groups according to age (ie; 21-30, 31-40, 41-50, 51-60, 61-70, 71-80, 81-90). There was increased incidence of Cervical cancer observed in the age group of 51-60years (32.8%) followed by 41-50 years (27.9%) and 61-70years (18.4%).

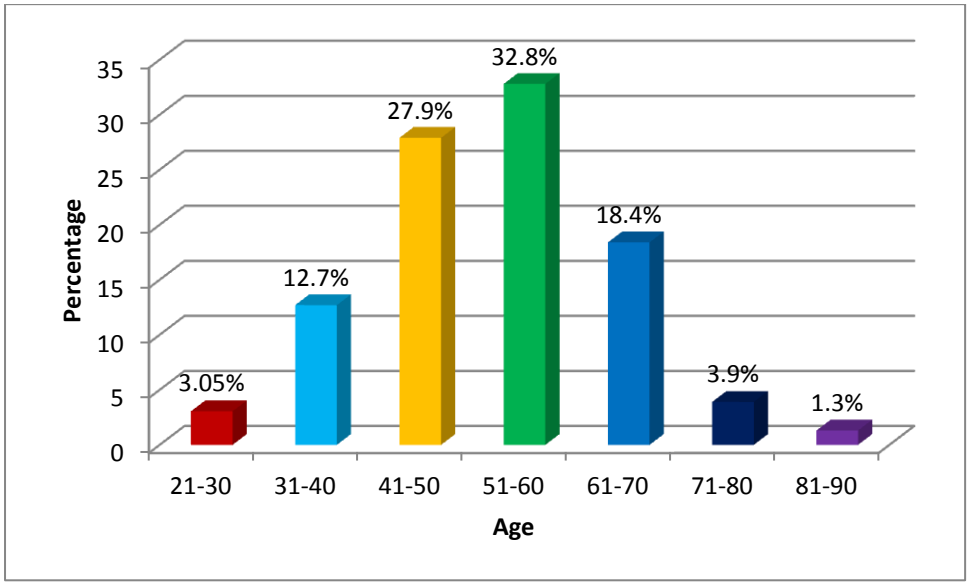
The age wise distribution of cervical cancers is given in the following Table-1 & chart-1

TABLE-1

S.NO	AGE GROUPS	NO OF CASES	PERCENTAGE
1.	21-30	7	3.05%
2.	31-40	29	12.7%
3.	41-50	64	27.9%
4.	51-60	75	32.8%
5.	61-70	42	18.4%
6.	71-80	9	3.9%
7.	81-90	3	1.3%

AGE WISE DISTRIBUTION OF CERVICAL CANCER,

CHART -1



Cervical Intra epithelial Neoplasia (CIN) occurs at a much lower age. One third of the cases being found in women less than 30 years old.[16] In the present study Cervical intraepithelial Neoplasia constituted about 0.4% of various lesions of cervix and most of the cases were in the age group 31-40yrs followed by 51-60yrs.

The age wise distribution of Cervical Intraepithelial Neoplasia is given in the following Table-2.

TABLE-2

S.NO	AGE GROUP	NO.CASES LSIL	NO.CASES HSIL
1.	21-30		
2.	31-40	4(CIN I)	1
3.	41-50	1(CIN I)	1
4.	51-60		2
5.	61-70	1*	1
6.	71-80		1
7.	81-90		
		0.2%	0.2%

1* condyloma acuminatum

The following Table -3 shows the distribution of cervical cancers according to differentiation.

TABLE-3

S.NO	GRADE	NO.CASES	Percentage
1.	Well differentiated	8	3.2%
2.	Moderately differentiated	192	91.8%
3.	Poorly differentiated	9	4.3%

In this study most of the cases are moderately differentiated (192cases 91.8%) followed by poorly differentiated squamous cell carcinoma (9cases 4.3%).Well differentiated carcinoma constitute 3.8% of total cases.

The following *Table-4* shows distribution of cervical cancers (squamous cell carcinoma) according to morphological subtypes

TABLE-4

S.No	Morphological subtypes	No of cases	Percentage
1.	Non keratinizing SCC	192	90.9%
2.	Keratinizing SCC	8	3.8%
3.	Small Cell SCC	9	4.3 %
4.	Papillary squamo-transitional carcinoma	3	1.4%

The following Table -5 shows distribution of Glandular and other cervical neoplasm :-

TABLE-5

S.No	Morphologic	No of cases	Percentage
1	Adenocarcinoma	10	4.3%
2	Clear cell adenocarcinoma	2	0.8%
3	Adeno squamous	3	1.3%
4	Adeno sarcoma	1	0.4%
5	Malignant melanoma	1	0.4%

In 229 cases of cancer cervix most of the cases are Non-keratinizing squamous cell carcinoma(192cases), Keratinising squamous cell carcinoma (8cases), Small cell(9)cases, Papillary squamo transitional(3cases), Adenocarcinoma(10cases). Clear cell adenocarcinoma(2cases), Adenosquamous(3cases), Adenosarcoma(1case) and Malignant melanoma (1 case).

In this study 10 cases of Non Keratinizing squamous cell carcinoma, 8 cases of Keratinizing squamous cell carcinoma, 9 cases of small cell Non Keratinizing squamous cell carcinoma, 8 cases of Adenocarcinoma were subjected to AgNOR study.

This study also included 5 control of normal cervical mucosa, 10 cases of chronic cervicitis with squamous metaplasia and 12 cases of cervical intraepithelial Neoplasia to evaluate AgNOR count in benign, premalignant and malignant lesions of cervix.

The present study was undertaken in the Department of Pathology, Coimbatore Medical College Hospital, Coimbatore.

A total of 62 cases of various lesions of cervix during the period August 2011-July 2012 were subjected to AgNOR study.

These were broadly classified into 8 groups according to their Histopathological reports as shown below in (Table 6.)

Table :6 Histopathological classification of cervical lesions :-

S.No	Groups	Subdivision
I	Histopathological normal cervical mucosa	Benign
II	Chronic cervicitis with squamous metaplasia	
III	LSIL (condyloma,CIN I,CIN II)	Premalignant
IV	HSIL(CIN III-Carcinoma in situ)	
V	SCC(well differentiated)	Malignant
VI	SCC (Moderately differentiated)	
VII	SCC (Poorly differentiated)	
VIII	Others*	

* includes adenocarcinoma and its variants.

The biopsy specimens which were received were subjected to routine paraffin sectioning at 3 μm thickness after proper fixation in 10% formal saline. AgNOR staining was performed by the procedure described[52] by Ploton et al .

The sections were deparaffinized in xylene and hydrated through decreasing grades of ethanol to double distilled deionized water.

The sections were then reacted with freshly prepared silver colloidal solution (1 part by volume of 2% gelatin in 1% formic acid and two parts by volume of 50% aqueous silver nitrate solution) for 35 min at room temperature, ensuring that a dark environment was maintained throughout the reaction time.

The silver colloidal solution was washed with double distilled ionized water, dehydrated through increasing grades of alcohol, cleared in xylene and mounted.

AgNOR Counting Procedure

The number of AgNORs which were present in each nucleus was counted in 100 nuclei by using a 100x oil immersion lens. At this magnification, AgNORs are visible both within and outside the nuclei [52]. The mean AgNOR values were calculated for each case and group. The results which were obtained in the counting procedure were analyzed statistically by using the Student's t-test, one way analysis of variance (ANOVA) for intergroup comparisons and Post hoc test -LSD (Least Significant Difference) for multiple comparison.

The mean AgNOR count of the studied groups was as follows **:**

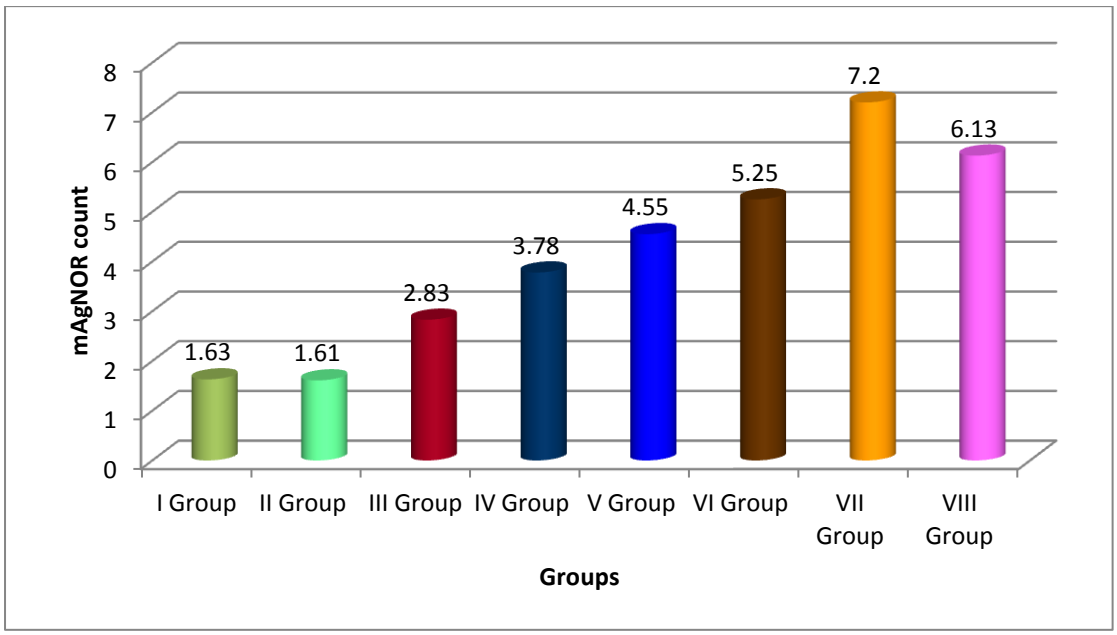
TABLE :7

Groups	No Cases	mAgNOR	SD
I	5	1.63	.10
II	10	1.61	.11
III	6	2.83	.19
IV	6	3.78	.16
V	8	4.55	.26
VI	10	5.25	.06
VII	9	7.20	.11
VIII	8	6.13	.04

The groups I & II (control subjects and benign lesions), could be differentiated clearly, based on the low value of the mAgNOR counts from the groups IV – VII. Group III and group IV differed from groups V-VIII, based on the value of the mAgNOR counts, which helped to demarcate between the pre-malignant and the malignant lesions of the

cervix and hence, helped to formulate the treatment plan for the patient, on whether a conservative approach or surgery was required.

mean AgNOR count in the studied groups- CHART 2



Groups V, VI and VII, VIII observed a significantly higher mean difference for the AgNOR count with all the rest groups. This indicated that squamous cell carcinomas of cervix and adenocarcinoma could be differentiated from the pre-malignant and benign lesions, based on the mAgNOR count also.

The total 37 cases of squamous cell carcinoma in our study were further graded into three grades according to the increasing number of the

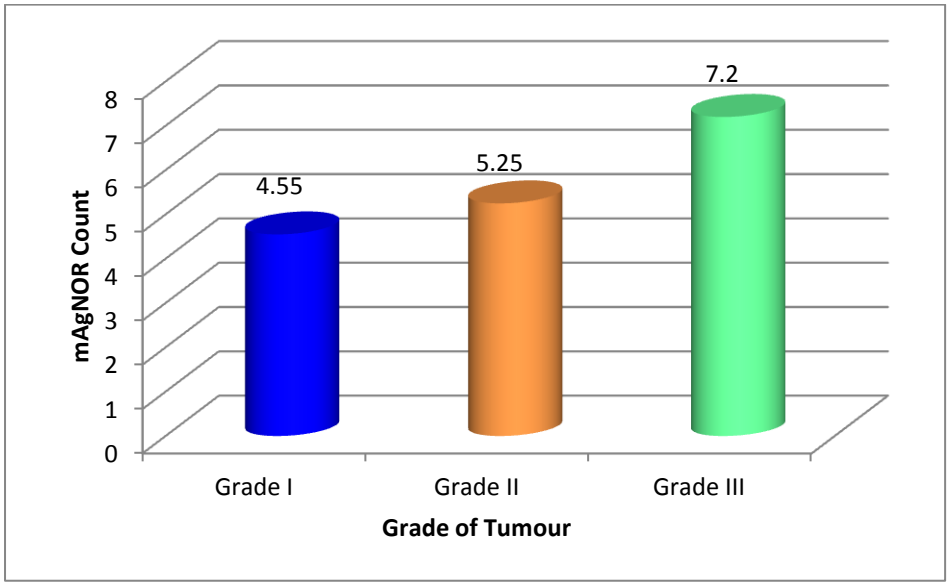
AgNOR dots (as shown in [Table8] and according to their morphology into well, moderately and poorly differentiated carcinomas.

Table :8 mAgNOR count according in different grades of tumor

Grade	No of cases	MAgNOR	S.D
I	8	4.55	0.26
II	20	5.25	0.06
III	9	7.20	0.11

The value of the mean AgNOR count for the three grades of squamous cell carcinoma of cervix in the present study were found to be 4.55 ± 0.26,5.25 ± 0.06 and 7.20 ± 0.11 respectively (as shown in [Table8]. The mean AgNOR count showed a linear and significantly increasing trend as the histopathological grade of the tumour increased (p<0.01)-chart -3 Study of mAgNOR count according to grade of tumor (squamous cell carcinoma)

mAgNOR count in different grades of tumour - CHART-3



In the present study, it was observed that the AgNOR dots were large, homogenously stained and regular in the nuclei of the normal cervical mucosa, chronic cervicitis with squamous metaplasia, whereas significant differences (irregular, giant and bizarre clusters) were seen in the squamous cell carcinomas.

In the malignant squamous cells, they appeared to be less uniform in size and shape and some dots appeared to be clumped together to form irregular bizarre shaped clusters ,which were more obvious in the moderately and poorly differentiated squamous cell carcinomas. Hence, by seeing the number and morphology of the AgNOR dots, squamous cell carcinomas of the cervix could be graded into well differentiated, moderately differentiated and poorly differentiated grades, which would

further help in the assessment of the prognosis of the lesion and hence, the outcome .

As shown in the [Table 9) intergroup comparison was done among 8 groups for the statistical analysis, which were divided by using the ANOVA test. The value was found to be statistically significant among the various comparative groups ($p < 0.01$) except between group I and group II.

There were no significant difference in AgNOR count between normal cervix and inflammatory lesions of cervix in the present study, hence insignificant p value ($p>0.05$).

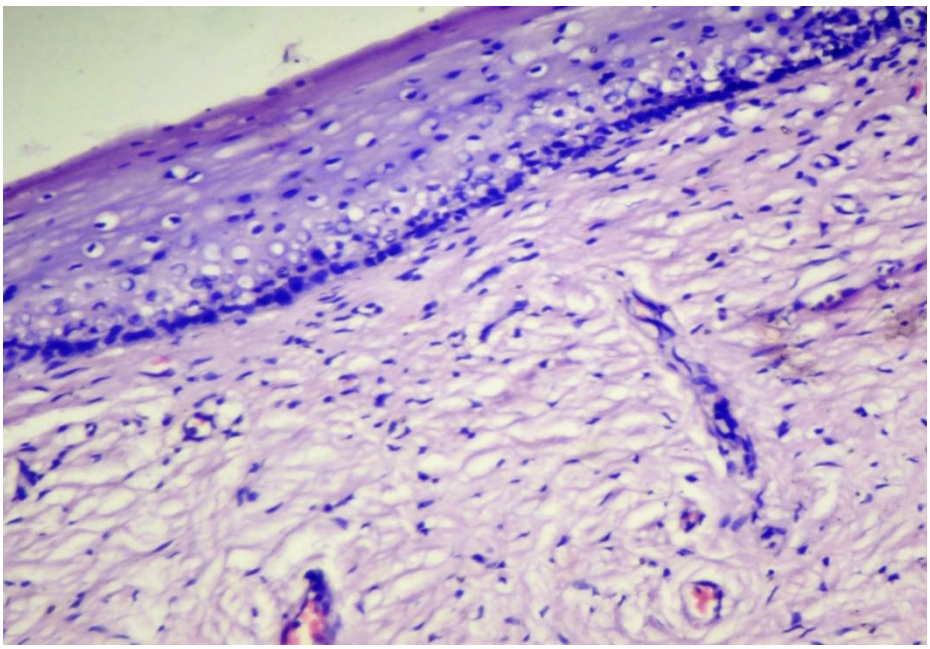
Comparison of mAgNOR count between various groups using ANOVA

TABLE - 9

Groups	Statistically significant difference with group	p value (less than)*	Not significant difference with group	p value (more than)
I	III,IV,V,VI,VII	<0.01	II	>0.05
II	III,IV,V,VI,VII	<0.01	--	--
III	I,II,IV,V,VI,VII	<0.01	--	--
IV	I,II,III,V,VI,VII	<0.01	--	--
V	I,II,III,IV,VI,VII	<0.01	--	--
VI	I,II,III,IV,V,VII	<0.01	--	--
VII	I,II,III,IV,V,VI	<0.01	--	--
VIII	I,II,III,IV,V,VI,VII	<0.01	--	--

* Significant p value

COLOUR PLATES



A: Normal squamous epithelium of ectocervix H&E STAINING (10X)

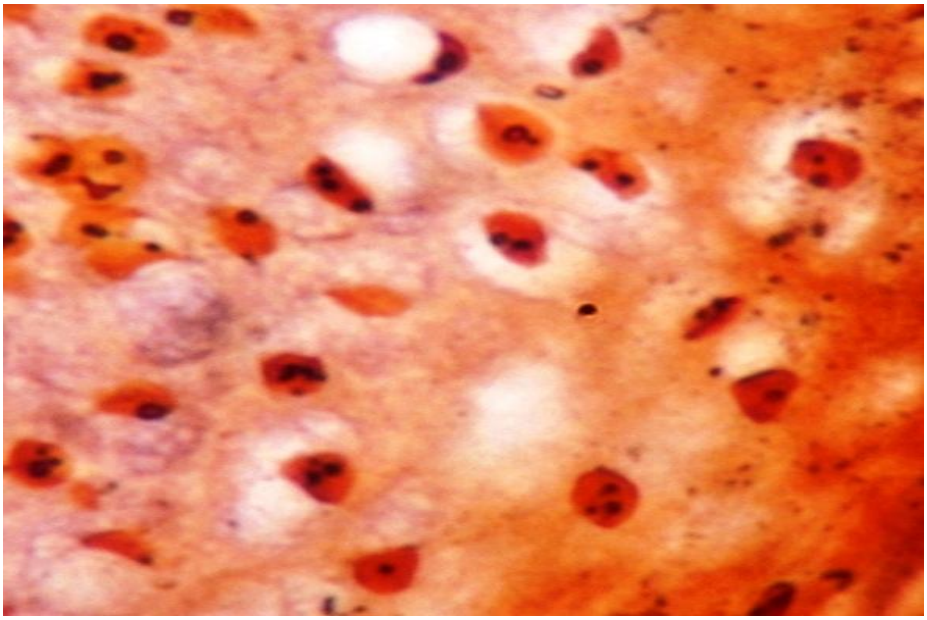


Fig 1B: AgNOR STAIN of Normal squamous epithelium of cervix mucosa showing homogenously stained and regular 1-2 AgNOR dots in the nuclei-(40x)

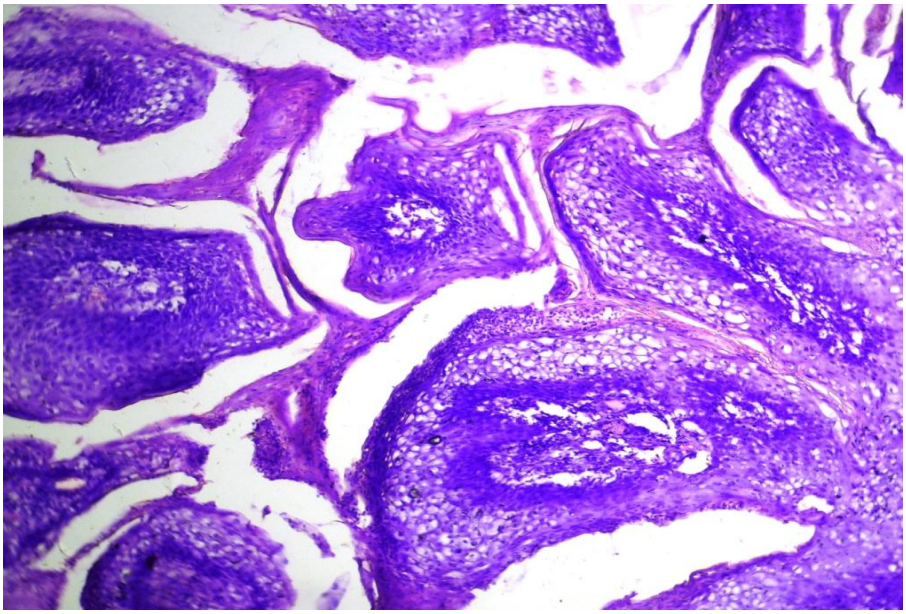


Fig 2A: Condyloma acuminatum showing superficial koilocytic changes (10X)

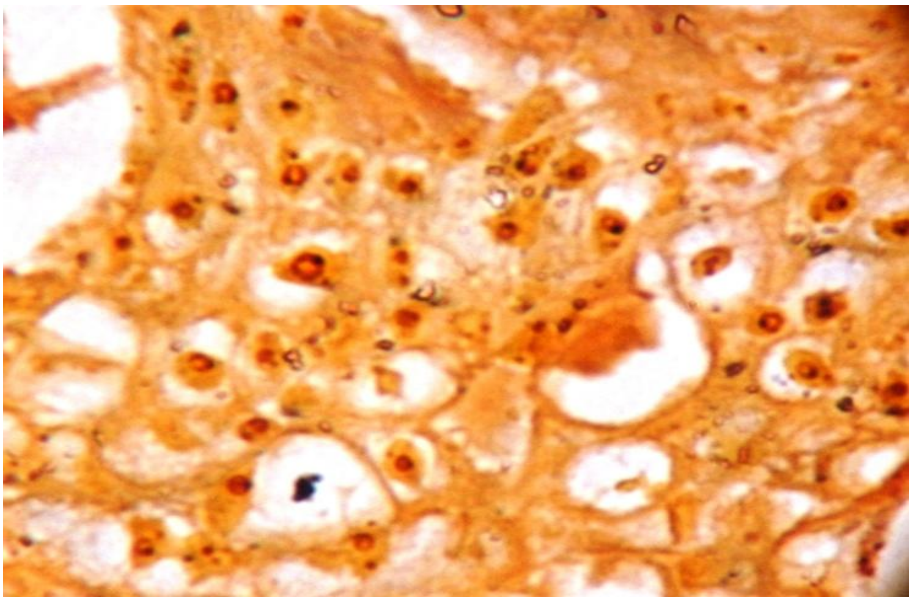


Fig 2B: AgNOR STAIN of condyloma acuminatum showing AgNOR dots in the nuclei(40X)

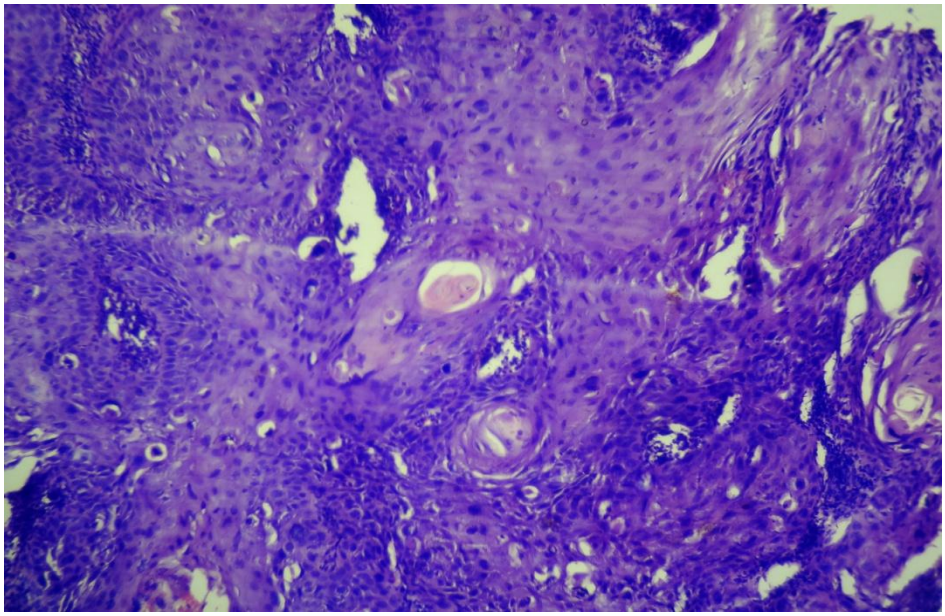


Fig 3A: Keratinising Squamous cell carcinoma -Well differentiated H&E (10X)

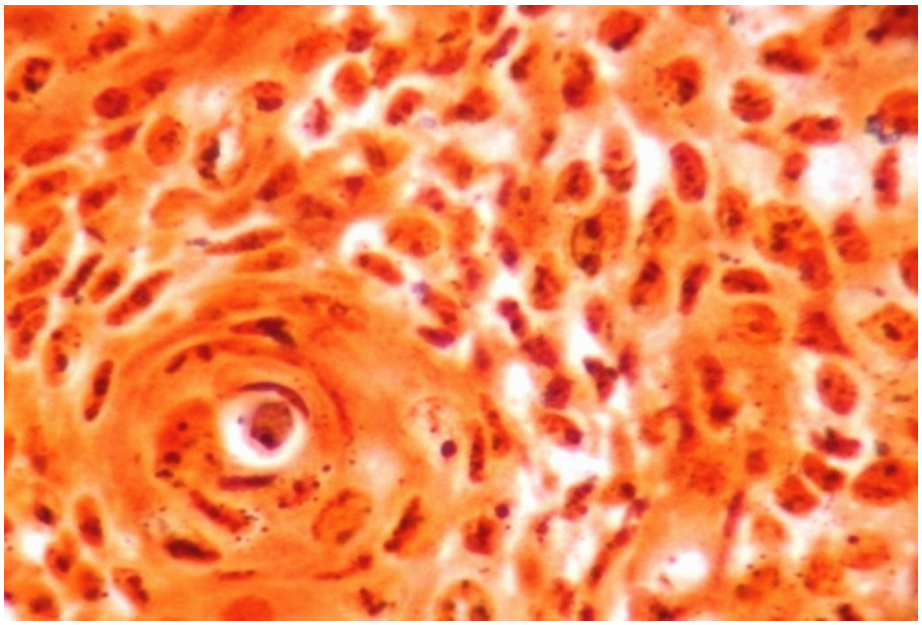


Fig 3B: Well differentiated squamous cell carcinoma - AgNOR STAIN (40x)

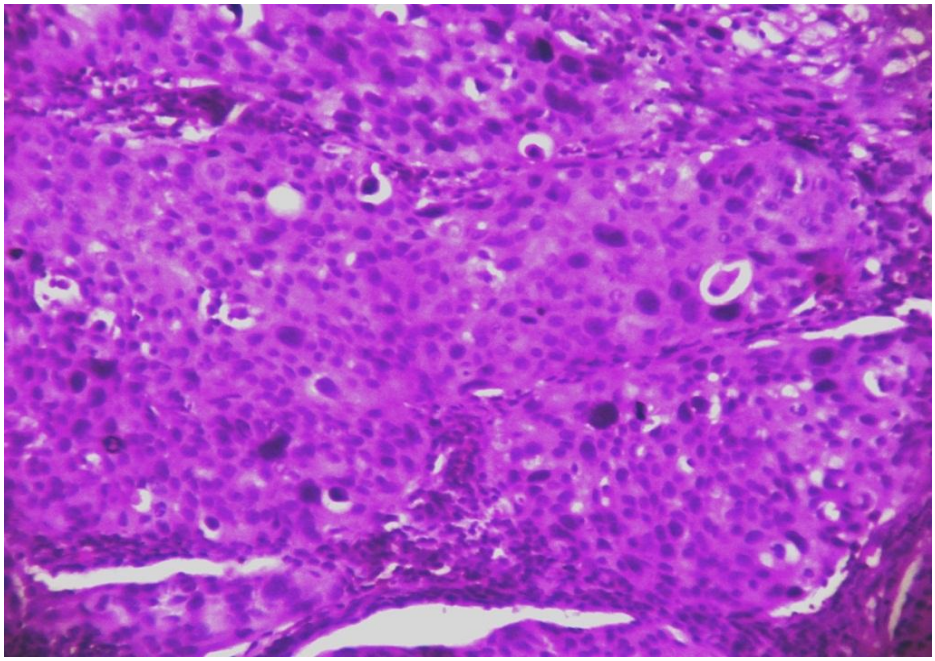


Fig 4A: Moderately differentiated squamous cell carcinoma H&E stain10X

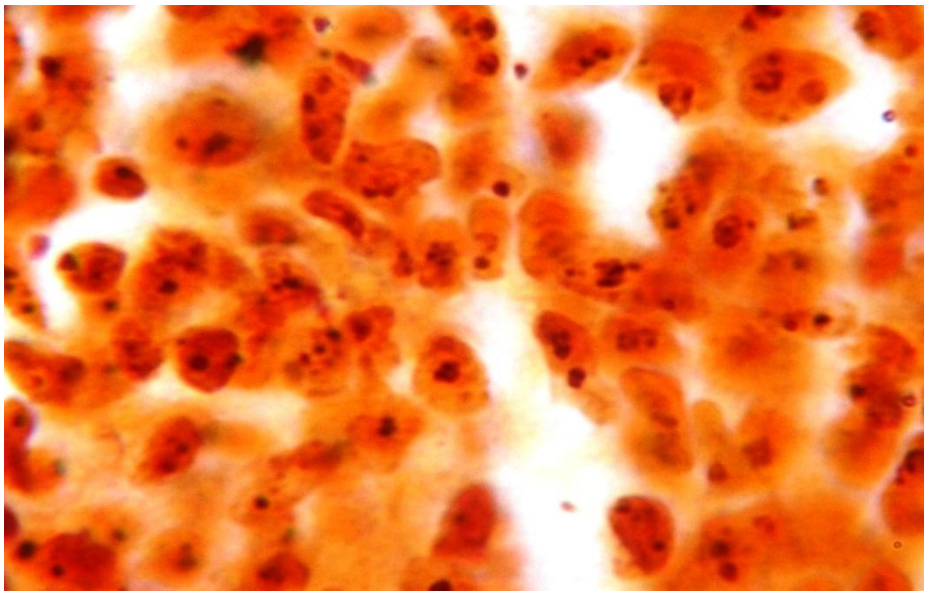


Fig 4B: AgNOR stain Moderately differentiated squamous cell carcinoma showing 3-4 AgNOR dots.

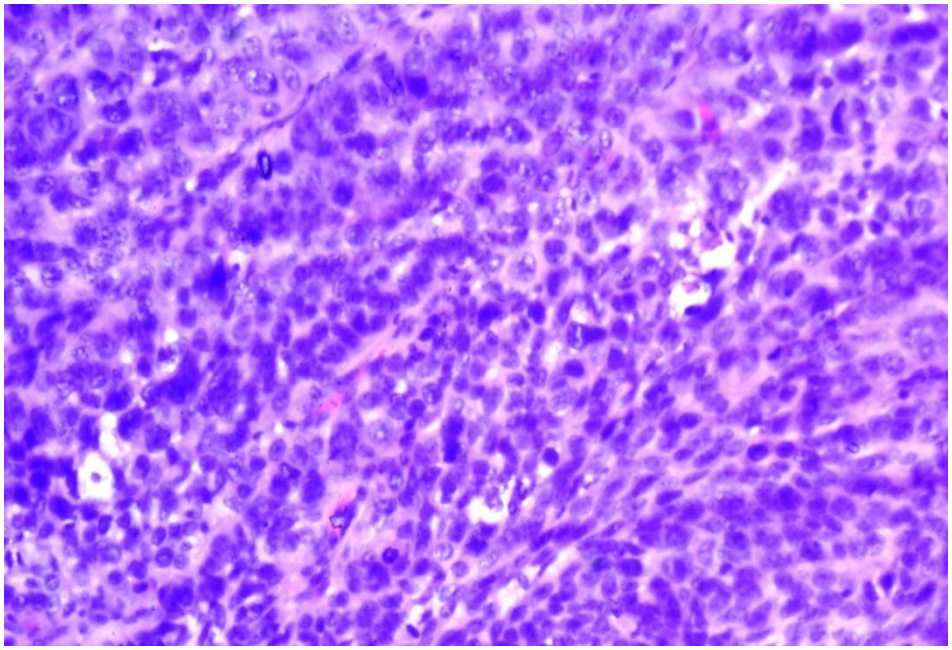


Fig 5A :Squamous cell carcinoma –poorly differentiated grade H&E (10X)

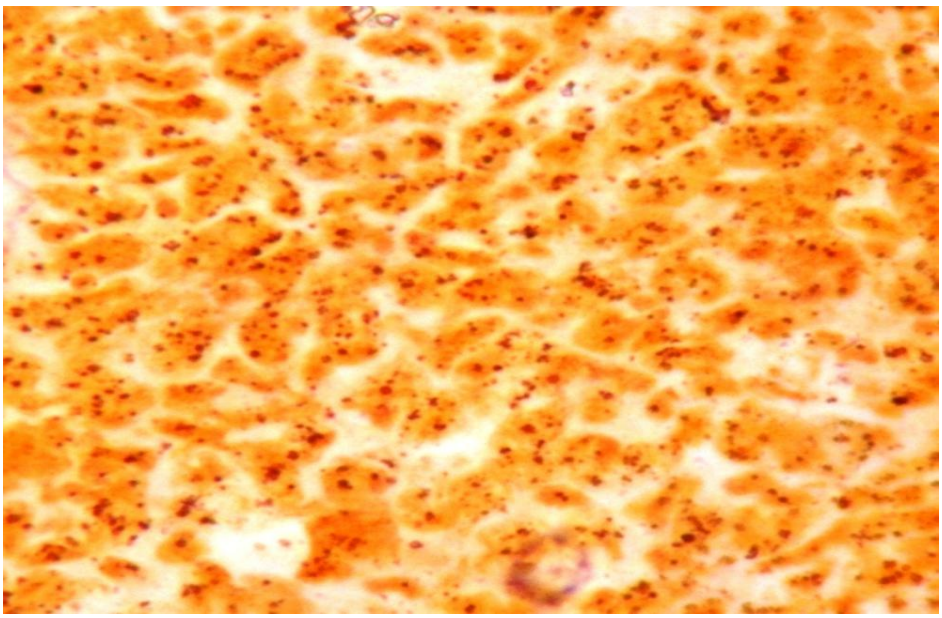


Fig 5B: Poorly differentiated squamous cell carcinoma showing numerous irregularly shaped AgNOR dots dispersed in the nuclei

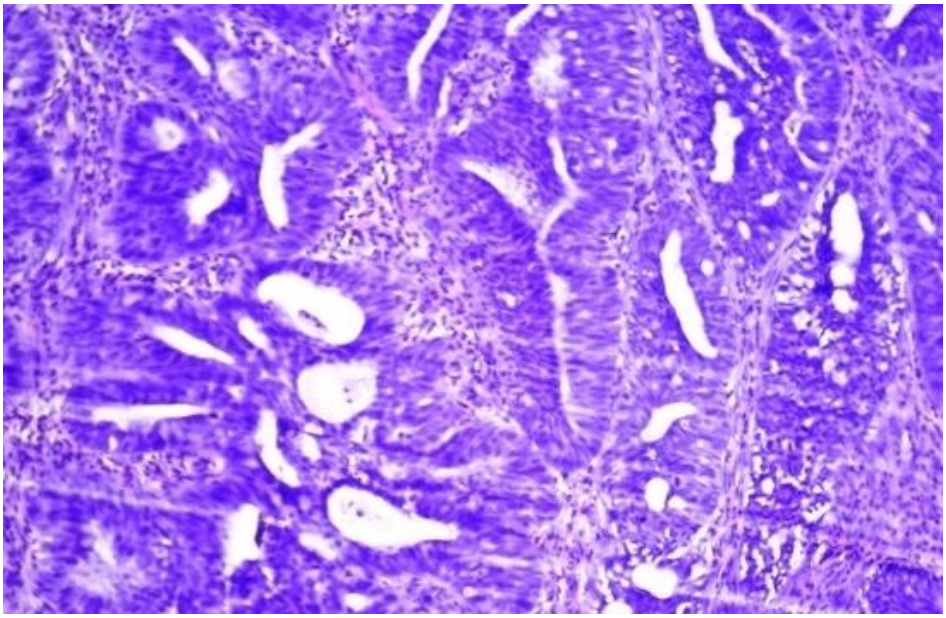


Fig 6A: Well differentiated Adenocarcinoma H&E (10X)

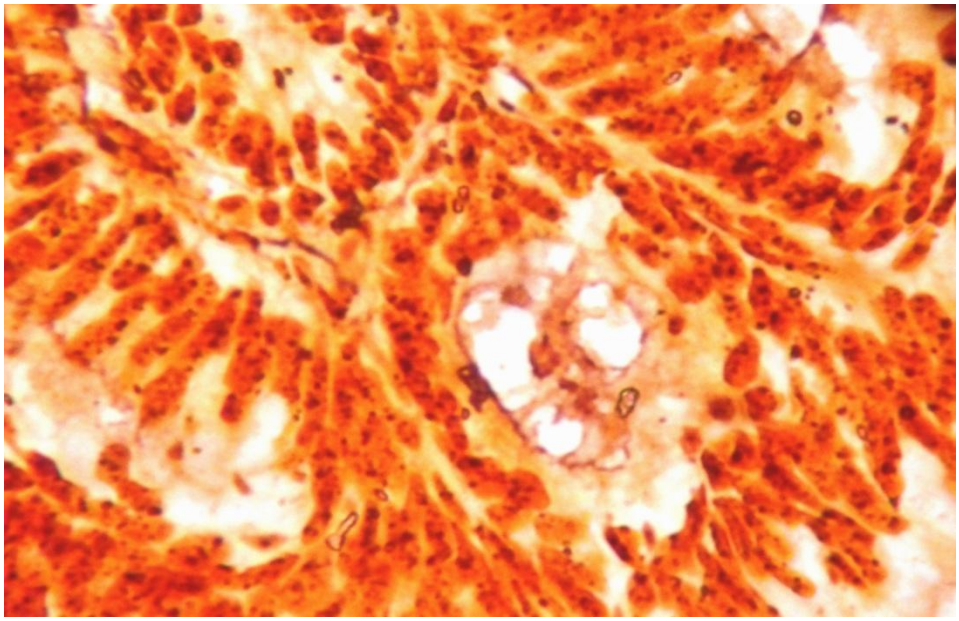


Fig 6B : AgNOR staining of well differentiated Adenocarcinoma showing numerous AgNOR dots

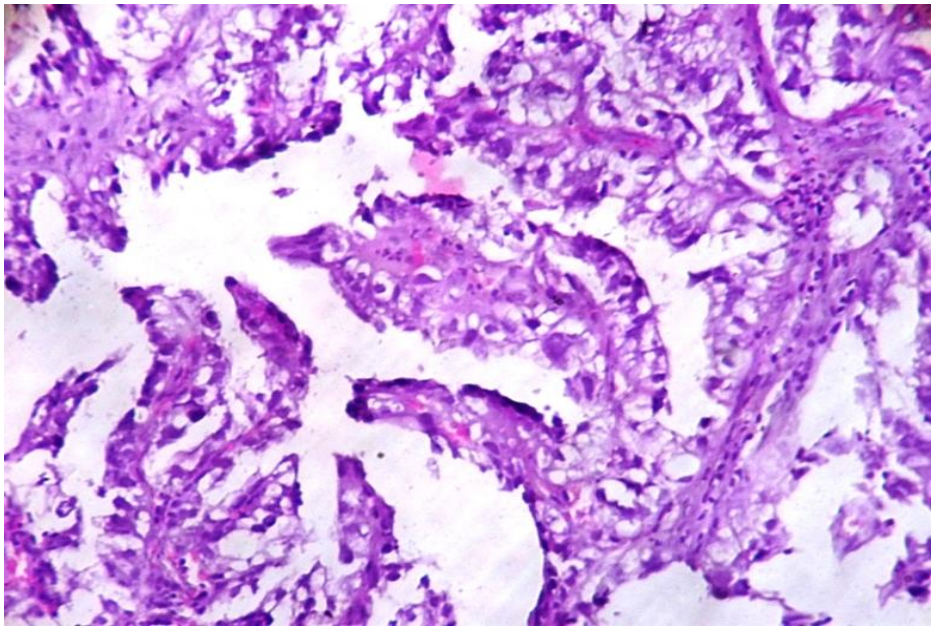


Fig 7A: Clear cell adenocarcinoma showing Hob nail cells H&E (10x)

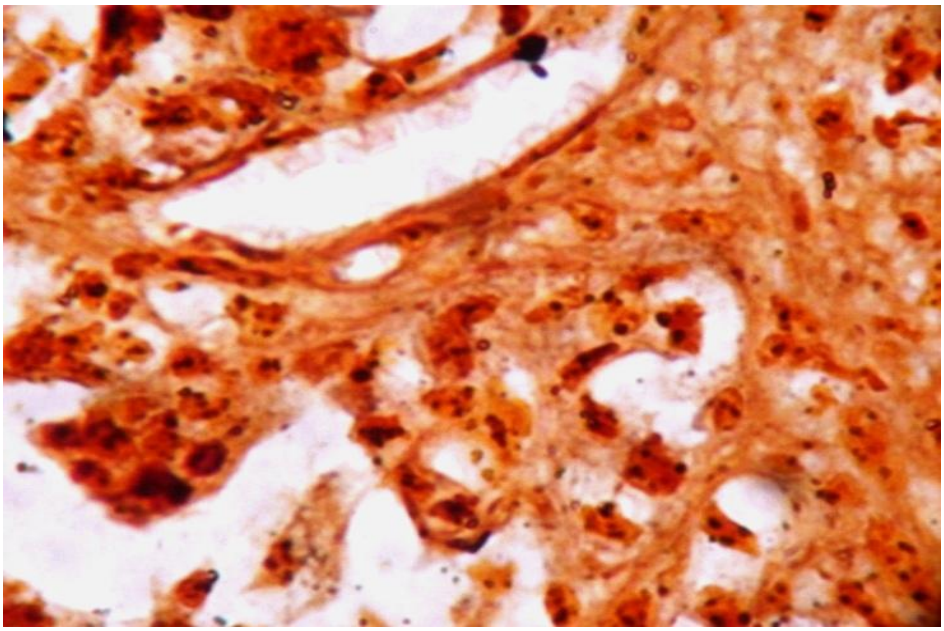


Fig 7B Pattern of AgNOR staining in Clear cell adenocarcinoma

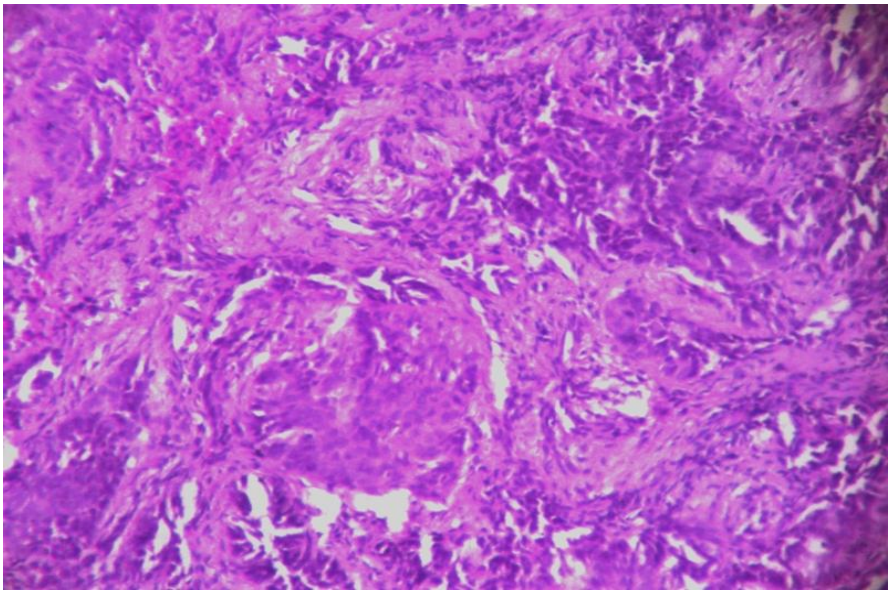


Fig 8A: Adenosquamous carcinoma showing both adeno and squamous epithelial components. (H&E-10X)

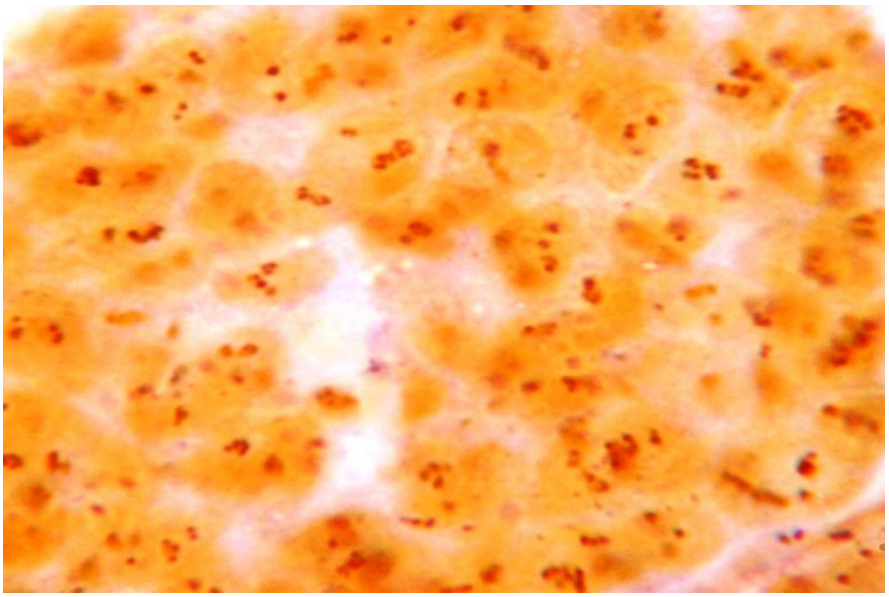


Fig 8B : Pattern of AgNOR staining in Adenosquamous carcinoma

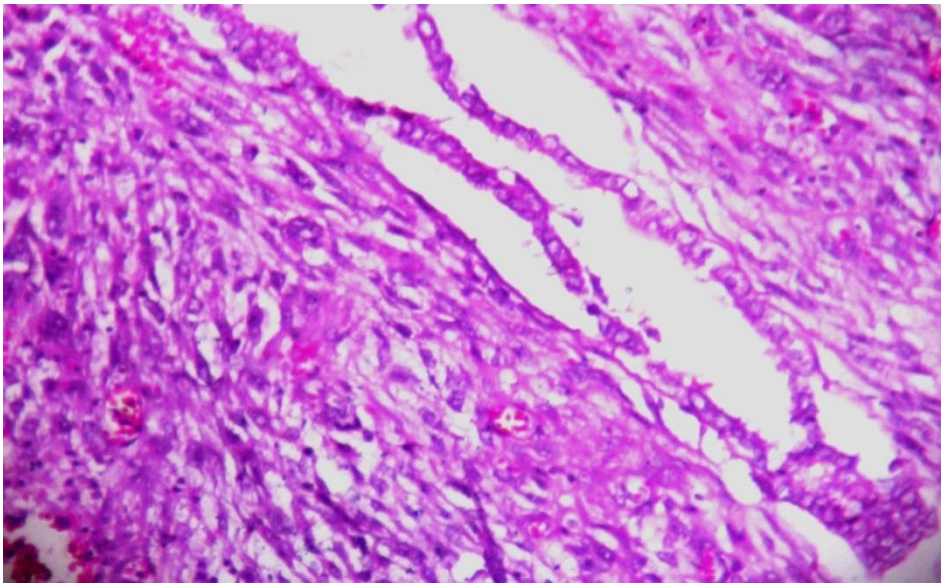


Fig 9A: Adenosarcoma-Biphasic tumour composed of benign glandular epithelial element and malignant stromal component

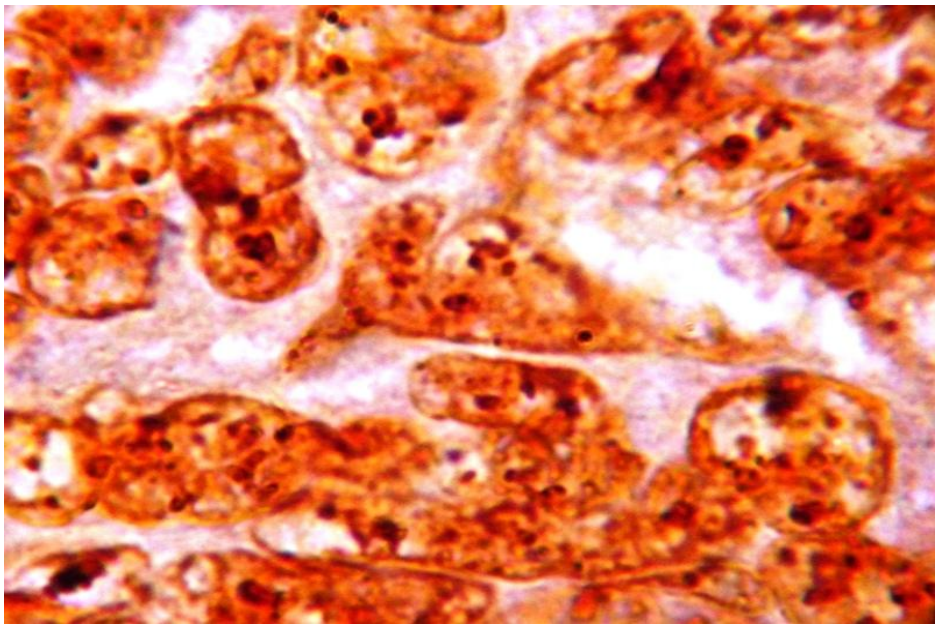


Fig 9B: Malignant stromal component of Adenosarcoma showing irregular bizarre and giant AgNOR dots

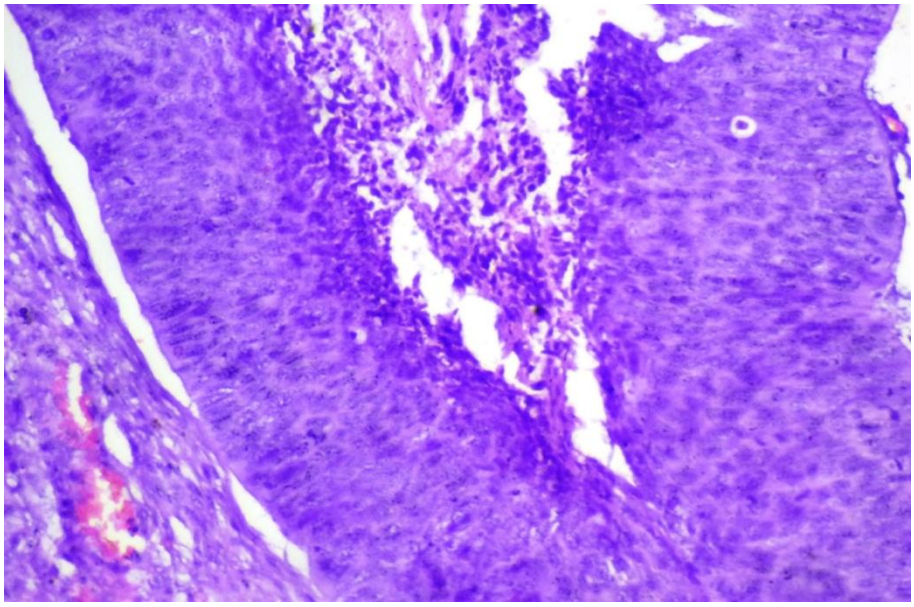


Fig 10A:Papillary squamo transitional carcinoma (H&E-10X)

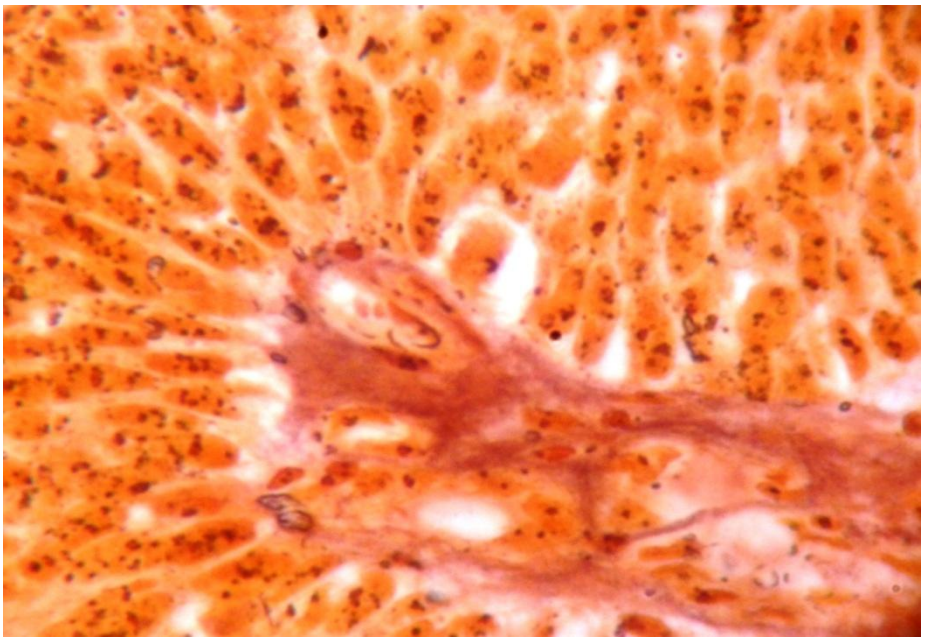


Fig10B: Pattern of AgNOR staining in papillary squamo transitional carcinoma

DISCUSSION

Cervical carcinoma is the second most common cancer women worldwide with increased mortality in about half of the new cases reported each year.

There has been increasing incidence of invasive cervical cancer and deaths from cancer cervix according to a recent estimate by cancer Society at America.

More than 80 percent of all cases of cervical cancer occur in developing countries.

Cervical cancer rates are lower in young women but increases with age. Due to the high prevalence of human papilloma virus infection around the age of 25 years the cancer incidence increases with age.

The risk of developing cancer cervix increases with age due to persistence of HPV infection.[42]

Routine effective screening before the age of 65 has reduced the incidence of cervical cancer .Above the age of 65 years cases of cervical cancer constitutes more than 20%.

In the present study the youngest age group at which cervical cancer was reported is 25yrs. Its incidence is common between 51-60 years followed by 41-50years.

According to the observation in literature the mean age for LSIL and HSIL was 3rd to 4th decade [68]

In the present study the mean age for LSIL and HSIL was 38 years and 53.3 years respectively .Data from the cancer registries indicate that more than 75% of cervical cancers develop in women above the age of 35 years[67].

In the field of tumour histopathology demonstration of nucleolar organizer regions (AgNOR) by one stage argyrophilic method is proving to be a useful staining procedure as a morphological indicator of prognosis.

The current phase of transcription is reflected by the size and the number and of NOR dots in the cells. It is significantly different in malignant cells from that in normal or benign cells. The NORs stains as black dots in the nucleus in the AgNOR method.

The squamous and columnar epithelial cells of cervix may show regenerative and repair changes following chronic infection and radiation therapy. They include marked cellular atypia with enlarged

hyperchromatic nuclei, coarse chromatin and macronucleoli. Irregular nuclear membrane and nuclear pleomorphism may be seen but absent mitotic activity.

These doubtful changes are sometimes misdiagnosed as cancer. The AgNOR technic proves to be a useful index of cell proliferation.

AgNOR method:

In this study, sections were cut at 3-4 microns thickness and fixed in 10% formalin. Visualization of the AgNOR dots were good with thin tissue sections.[54,55]

AgNOR counting: -

In this counting method, the number of interphase AgNORs which appear as well defined black dots are evaluated under light microscope. Each dot corresponds to one interphase NOR as visualized by electron microscopy.

Using the method suggested by Crocker et al[56,62] enumeration of AgNOR dots were done.Sampling of tissue, processing and staining procedures greatly influences evaluation of AgNOR dots. It is highly

subjective and only solution to these problems is by standardization of the procedure.

Another choice for Agnor quantification is by image cytometry which has high degree of precision, reproducibility, with minimal inter-observer errors. Cytogenetics which applied the AgNOR staining technique earlier has gained importance now as an indicator of proliferative activity of the cell.

AgNORs are usually indiscernible in normal cells because of the tight packing of nucleoli in the nucleus. Dispersion of individual AgNOR's occurs in rapidly proliferating neoplastic cells due to nucleolar disaggregation.

The present study was conducted to evaluate the importance of AgNORs in differentiating benign, pre-malignant and malignant lesions of the cervix.

For routine evaluation of cell kinetics for prognostic purposes, interphase AgNOR quantification appears to be very interesting and promising method.

In routinely processed samples it is the only method which permits information to be obtained on the rapidity of cell proliferation. The method more frequently employed involves evaluation of the sample

directly at the light microscope and counting the number of interphase AgNOR per cell. By carefully focusing throughout the section thickness at very high magnification (100 x oil immersion level) AgNORs are visible as black dots inside the nucleus. Numerous AgNOR dots were seen in poorly differentiated tumours.

Crocker et al who have done intensive work on NOR's in various tumors have observed three main types of AgNOR configurations in normal and neoplastic cells.[56] .

The following are the NOR patterns observed

- The NOR's are fully aggregated to form a solitary rounded structure,often seen in resting cell.
- Nucleolar pattern in proliferating cells, where NOR's can be seen within the nucleus.
- Dispersion of small NOR's throughout the nucleoplasm as frequently observed in highly malignant cells.

It was observed in the present study that the AgNOR dots were large and regular in the nucleus of the normal cervical mucosa and chronic cervicitis whereas irregular and giant were seen in squamous cell carcinomas and adenocarcinoma of cervix.

The mean AgNOR count of the normal cervical mucosa in the present study was 1.63 ± 0.10.The values are comparable with previous studies which were done by Prathiba et al (1.2), Ramsden et al (1.31), *Kafil* Akhtar (1.81).

Observation of AgNOR count by Cullimore *et al*[57] in normal squamous epithelium correlated well with the present study.

KC Shiva Raj et al [40]evaluated the role of AgNOR in differentiating benign and premalignant lesions from malignant lesions in both cervical smears and histology in 53 cases.

They found that in all benign lesions like chronic cervicitis and chronic cervicitis with squamous metaplasia AgNOR counts were less than 2. In low grade squamous intraepithelial lesions AgNOR counts fall between 2.22 and 3.26 in cervical smears whereas in high grade intraepithelial lesions it was between 3.16 and 3.70 . In carcinoma cases it ranged from 4.5 to 5.58.

In the present study the mAgNOR counts for chronic cervicitis with squamous metaplasia, LSIL, HSIL was 1.61±0.11, 2.83±0.19 and 3.78±0.16 respectively.

Significant increase of AgNOR counts was noted from CIN-I to CIN-III to carcinoma cervix but not significant difference in AgNOR

counts was noted between normal cervix control and chronic cervicitis with squamous metaplasia. This observation was in accordance with the observations of Egan *et al* [36].

AgNOR shape also varied in benign lesions, precancerous and malignant cases. In LSIL; the majority of the dots were homogenous symmetric and had regular contours. In case of carcinoma, the dots were asymmetric and had irregular contours. They were aggregated, smaller and more scattered whereas, in HSIL the AgNOR dots morphology were having intermediate picture. The percentage of aggregated dots and scattered dots increases as the disease progresses. [40]

Egan, et al [36] observed that mean AgNOR count increased steadily whereas the mean size of AgNORs decreased from CIN I to CIN III.

Cardillo [34,65] studied AgNOR counts in cervical smears of squamous metaplasia and cervical intraepithelial neoplasia. Previously stained Papanicolaou smears were destained and restained with AgNOR silver.

In the present study statistically significant difference ($P<0.05$) in AgNOR counts were found in squamous metaplasia and various grades of CIN.

An Indian study done by Pratibha and Kuruvilla[24] on the role of AgNOR in diagnosis of premalignant and malignant lesions of the cervix, showed there was a progressive increase in mean AgNOR count from normal to CIN I, CIN II, CIN III and invasive carcinoma.

The difference between counts in CIN I and II and in normal cervix and between counts in CIN III and in invasive carcinoma in this study was statistically significant [24].

In the present study it was also noted that the size of AgNOR dots decreased with increase in AgNOR count. This is in accordance with the study reported by Egan et al [36] who noted an inverse relationship between AgNOR numbers and sizes, and proved that CIN III could be distinguished from CIN I and II on the basis of AgNOR sizes. AgNOR counts can thus be useful in differentiating doubtful cases of CIN.

AgNOR counts also have prognostic significance. It has been noted that CIN lesions with low AgNOR counts are more likely to regress in comparison to CIN lesions with high AgNOR counts[36]

However Rowland [37] in a study on nucleolar organizing regions in cervical intraepithelial neoplasia, did not find any significant difference in AgNOR count in squamous epithelium of normal cervix, CIN I and CIN II but there was a small but significant increase in CIN III group.

Although there is variation in AgNOR counts in different studies the difference is statistically signficant in various grades of CIN in all studies except in the study reported by Rowlands [37].

S. Lakhmi et al [44] in their study observed a significant positive correlation between AgNOR counts and tumor progression .They evaluated AgNOR counts in inflammatory lesions of the uterine cervix, cervical intra-epithelial neoplasia, and invasive cervical squamous carcinoma. Significant differences in AgNOR counts were observed between the three study groups, with maximum counts in invasive carcinoma of cervix.

In the present study AgNOR counts were also compared in the different grades of squamous cell carcinoma of the cervix. A progressive significant increase in the counts was observed with increasing grades of carcinoma.

Counts of 4.55 ± 0.26 were recorded in well-differentiated carcinoma while moderately and poorly differentiated carcinomas yielded counts of 5.25± 0.06 and 7.20 ± 0.11 respectively. The difference was statistically significant ($P < 0.01$) between well differentiated, moderately differentiated and poorly differentiated carcinomas. These findings strongly support the view that proliferative activity and malignant potential of neoplastic lesions of the cervix increase progressively as the

grade of the lesion becomes higher. Similar statistically significant AgNOR scores were obtained by Miller et al[64] in cases of well differentiated squamous cell carcinoma of the cervix and poorly differentiated cases.

Prathiba and Kuruvilla[24] reported a mean AgNOR count of 4.3 in large cell keratinizing type (moderately differentiated) squamous cell carcinoma whereas AgNORs in the small cell type (poorly differentiated) were dispersed as very fine dots making the score difficult to ascertain.

Their study also recorded that AgNOR count increased with increasing grade of malignancy while Newbold et al[58] failed to demonstrate any association between tumor grade and AgNOR count.

However, Agarwal and Gupta[46] have reported a mean AgNOR score of 5.27 ± 0.10 and 5.41 ± 0.72 in well differentiated and poorly differentiated squamous cell carcinoma respectively. This is not in agreement with the present observation.

Pahuja S et al[51] in their study analysed the cellular proliferative activity in squamous intraepithelial and invasive lesions of cervix in 50 cervical biopsies. They found that amongst invasive malignancies, highest mean of AgNORs per nucleus was observed in poorly differentiated squamous cell carcinoma of cervix. In conclusion,

AgNORs can prove to be a simple inexpensive and reliable proliferation marker in lesions of cervix.

Kafil Akhtar et al [47] in their study subjected 50 histologically proven, previously untreated cases of various grades of squamous cell carcinoma of the cervix to Cobalt-radiotherapy .They found that AgNOR scores could differentiate between different grades of malignancy, high counts indicating a higher grade, while significant decline in AgNOR counts after radiotherapy denotes a good prognosis.

AgNOR is an effective tool reflecting the proliferation rate of the tumor and has a significant diagnostic and prognostic value in tumor pathology

According to *Murty et al*[59] in carcinoma cervix not only the number of NoRs become more prominent and larger in size, many times appearing giant size . This finding was in accordance to the present study.

There was also a significant increase in NOR from grade I to grade III Carcinoma. This correlated well with the study by *Murty et al*[59]. Degree of differentiation and transcriptional activity[60] was reflected by AgNORs position particularly ectopic AgNOR or abnormal distribution.

During this study several technical problems interfered with the staining method .They are duration of the staining, the temperature, the

purity of water and the reagents. The above factors influenced the final result to a great extent.

Particular attention was paid to the cleanliness of the glassware and the purity of water in order to avoid background staining and non-specific granular deposits on the tissue sections [69].

With adequate efforts made to standardize the tissue processing and the staining techniques the silver staining technic in diagnostic pathology has achieved a new milestone. [53]

The AgNORs have been shown to reflect DNA transcriptional activity. Study of AgNORs has been identified as a reliable indicator of cell proliferation and in turn, of the malignant potential of a lesion [24]. Malignant tumor cells are characterized by extremely large AgNORs, which show a random or scattered distribution. They are useful in discriminating between benign and malignant conditions being significantly higher in neoplastic cells than in normal cells [61,63]. They also serve as a significant prognostic indicator in malignant lesions [44].

The AgNOR technic which was earlier used extensively in cytogenetics has now gained importance as an indicator of cell proliferation. AgNOR scores differentiate between different grades of

malignancy, high counts indicating a higher grade, while significant decline in AgNOR counts after radiotherapy denotes a good prognosis.

A correlation between the AgNOR count and prognosis was too found in colorectal cancer, benign and malignant effusions, adenoid cystic carcinoma and breast carcinoma. It is of prognostic value also in ovarian cancer transitional cell carcinoma of the bladder and glottic cancer.[32]

CONCLUSION AND SUMMARY

1. The incidence of cancer cervix is 19% of all malignancies in various organs received for histopathological examination at Coimbatore medical college Hospital.
2. Squamous cell carcinoma of cervix constitutes 92.5% of cervical cancers .Most of the cases were Non-keratinizing squamous cell carcinoma and Moderately differentiated grade.
3. Cervical cancers are common between the age group of 51-60 years
4. In the present study, it was observed that the AgNOR dots were large, homogenously stained and regular in the nucleus of the normal cervical mucosa and chronic cervicitis with squamous metaplasia whereas significant differences (irregular, giant and bizarre clusters) were seen in squamous cell carcinomas and adenocarcinoma of cervix

The misery of women due to cervical cancer, which is the commonest cancer in women in a developing country like ours is a scourge of humanity.

Early diagnosis and management of squamous intraepithelial lesions (SIL) is the best way to achieve control over cancer cervix.

Of the various newer techniques based on nuclear studies, assessing the tumour by staining of AgNORS using a silver compound has become popular for its

- Simple procedure
- Easy use
- Reasonable Cost
- Correlation with other markers of proliferation

The present study also shows an inverse relationship between AgNORs number and their size . There was a continuous increase in AgNORs from normal to carcinoma along with decrease in size of individual AgNORs.

As a marker of cellular activity as well as malignancy, AgNOR count can be utilized for discriminating benign, precancerous and cancerous lesions. It is also useful in differentiating the atypical changes in regenerating epithelium, postmenapausal atropic mucosa, and following radiation therapy. These lesions are usually misdiagnosed as cancer by histopathological examination.

AgNOR count is a reproducible simple efficient and inexpensive method which can be used as an adjunct to routine cytology and

histopathology for diagnosis of Cervical Intraepithelial Neoplasia especially in doubtful cases.

Studies have reported that dysplasia with low AgNOR counts are more likely to regress as compared to those with high AgNOR counts which on the other hand are likely to progress to invasive cancer showing its prognostic significance.

Thus, the AgNOR technique can be useful as a supportive tool in the prognosis and therapeutic decision in squamous cell carcinomas to the routinely performed haematoxylin and eosin staining.

The staining procedure is simple and cost effective. But to achieve good results a lot of dedication, standardization and meticulous bench work is needed.Therefore AgNOR quantify can be considered only as one, among the other well established cyto-histopathological parameters to be used for diagnosis of malignancy.

In conclusion, AgNORs can prove to be a simple inexpensive and reliable proliferation marker in lesions of cervix and can be a used as a supplementary factor for the difficult histoprognostic evaluation of various malignancies.

DEPARMENT OF PATHOLOGY

COIMBATORE MEDICAL COLLEGE AND HOSPITAL

COIMBATORE

PROFORMA

IP NO: HPE NO :

Patient Name :

Age: IP/OP : Religion & Caste:

Occupation: S.E. Status:

Address:

Clinical diagnosis:

1) **PRESENTING COMPLAINTS**:

A) Bleeding per vagina

Amount : Heavy( ) Moderate( ) Scant( ) Variable( )

Duration: Intervals.

Episodes of intermenstrual bleeding : Yes ( ) No ( )

Duration: Days.

B)History of dysmenorrhoea : Yes( ) No( )

C) History of White discharge : Yes( ) No( )

II) **OBSTETRIC HISTORY**:

Married Life: Para:

Abortion : Yes( ) No( ) No of Abortion:

Last Delivery : Tubectomy : Yes( ) No( )

III) MENSTRUAL HISTROY:

Age of Menarche: Years.

Menstrual Cycle : Regular ( ) Irregular ( )

Duration of the cycles: Days

Amount of Bleeding : Heavy( ) Moderate ( ) Scant( )

History of dysmenorrhoea :Yes( ) No( )

Others :

Previous Menstrual History :

Interval : Days ,Duration of bleeding :

Amount : Heavy( ) Moderate( ) Scant( ) Variable( )

History of Dysmenorrhoea : Yes( ) No( )

History of white Discharge : Yes ( ) No( )

Last Menustrual Period :

IV) **PERSONAL HISTORY** :

Appetite : Good( ) Reduced( ) Variable( )

Bowel Habits : Regular( ) Irregular( ) Variable( )

Micturition frequency

Burning Micturition : Present( ) Absent( )

Other Abnormality :

Vegetarian ( ) Non vegetarian ( ) Mixed ( )

V**) HISTORY OF ANY DISEASE**:

History of Tuberculosis : Yes( ) No( )

History of Diabetes Mellitus : Yes( ) No ( )

History of Hypertension : Yes( ) No ( )

History of Endocrine Disease (Thyroid) : Yes( ) No ( )

History of Bleeding Disorder: Yes( ) No ( )

History of any other major illness (specify) :

History of any Drug intake (specify):

VI**) FAMILY HISTORY** :

Diabetes ( ) Tuberculosis ( ) Hypertension( )

Obesity( ) Malignancy ( )

Other (specify):

VII) **GENERAL EXAMINATION** :

Built and Nourishment : Poor ( ) Moderate ( ) well( ) Obese( )

Height : Weight : Pulse /Min

Blood Pressure : mmHg. Anaemia : Yes( ) No( )

Jaundice : Yes ( ) No( ) Lymphoadenopathy: Yes( ) No( )

Oedema : Yes( ) No ( )

VIII) **SYSTEMIC EXAMINATION**:

Cardiovascular System :

Respiratory system :

Per abdomen :

Liver : Enlarged( ) Normal ( )

Spleen : Enlarged ( ) Normal( )

Operation Scar : Present ( ) Absent( )

Mass : Palpable ( ) Not Palpable( )

Other (specify)

Breast :

Thyroid :

Skin :

Central Nervous System :

IX) Local Examination

Vulva : Healthy ( ) Unhealthy ( )

Other (specify)

Bleeding : Present( ) Absent ( )

Per Vaginal Examination:

Cervix : Direction :

Consistency :

Bleeds on Touch : Yes( ) No( )

Cervical Erosion : Present( ) Absent ( )

Other (specify):

Uterus : Anteverted ( ) Retroverted( )

Normal Size ( ) Bulky ( ) Small ( )

Mobile ( ) Fixed ( )

X) LABORATORY INVESTIGATIONS:

1) URINE : Albumin:

Sugar :

Microscopy:

Other Abnormalities (If any):

2) HEAMATOLOGY :

Heamoglobin: gms/dl .

RBC Count : Millions /cu.mm.

Total Leukocyte Count : / cu.mm

PCV : % , MCV: fl

MCH : pg MCHC gms %

Differential Count :

Platelet count : /cu.mm E.S.R ; mm/hr

Blood Group & Rh Typing :

Bleeding Time : Clotting Time :

Peripheral Blood Picture :

3) **BIOCHEMICAL TESTS**:

Random Blood Glucose : mg/dl

Blood Urea : mg/dl

4) **RADIOLOGICAL INVESTIGATIONS**:

Ultrasonographic findings:

Others(specify):

5) **BIOPSY** :

(Diagnostic Dilatation & Curettage Of Endometrium

Finding during D&C (if any)

Cervix Biopsy :

Gross Appearance:

Microscopic Appearance:

METHOD OF HEMATOXYLIN AND EOSIN STAINING

Reagents used;

1. Erhlich s Hematoxylin solution

2. Eosin Y solution 1%

3. Acid Alcohol solution 1%

Procedure:

1.Deparrafinize the sections

2.Immerse the sections in xylene for 30 minutes

3.Then place in isopropyl alcohol for 15 minutes

4.Wash in tap water

5.Stain the sections with Erhlich s Hematoxylin or 10-15 minutes

6.Wash in tap water

7.Differentiate in 1% acid alcohol solution-2 to 3 dips

8.Blueing for 10 minutes

9.Counterstain with 1% eosin solution 2-3 dips

10.Wash in tap water

11.Air dry the sections

12.Xylene -Mount

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MASTER CHART

S NO	HEP NO	AGE	SEX	IP NO	SPECIMEN TYPE	HPE DIAGNOSIS	AgNOR COUNT	mAgNOR COUNT	SD
1	G1124/11	54	F	205321	HYSTERECTOMY	C X- NORMAL HISTOLOGY	1.5	1.63	0.1
2	G1132/11	50	F	216689	HYSTERECTOMY	CX -NORMAL HISTOLOGY	1.6		
3	G1155/11	45	F	230519	HYSTERECTOMY	CX -NORMAL HISTOLOGY	1.6		
4	G1158/11	52	F	232129	HYSTERECTOMY	CX-NORMAL HISTOLOGY	1.75		
5	G1163/11	49	F	207803	HYSTERECTOMY	CX-NORMAL HISTOLOGY	1.7		
6	G1245/11	44	F	229485	CERVIX BIOPSY	CRC WITH SM	1.5	1.61	0.11
7	G1284/11	50	F	235843	CERVIX BIOPSY	CRC WITH SM	1.6		
8	G1295/11	53	F	229718	CERVIX BIOPSY	CRC WITH SM	1.6		
9	G1298/11	46	F	38785	CERVIX BIOPSY	CRC WITH SM	1.75		
10	G1334/11	48	F	252250	CERVIX BIOPSY	CRC WITH SM	1.7		
11	G1431/11	58	F	253975	CERVIX BIOPSY	CRC WITH SM	1.4		
12	G1477/11	56	F	251586	CERVIX BIOPSY	CRC WITH SM	1.7		
13	G1493/11	43	F	265880	CERVIX BIOPSY	CRC WITH SM	1.6		
14	G1530/11	49	F	274184	CERVIX BIOPSY	CRC WITH SM	1.7		
15	G1542/11	54	F	49827	CERVIX BIOPSY	CRC WITH SM	1.5		
16	G1873/11	35	F	408170	CERVIX BIOPSY	LSIL	2.6	2.83	0.19
17	G1979/11	38	F	396951	CERVIX BIOPSY	LSIL	2.6		
18	G2024/11	40	F	46640	CERVIX BIOPSY	LSIL	2.9		
19	G2041/11	36	F	47542	CERVIX BIOPSY	LSIL	2.9		
20	G2066/11	48	F	458487	CERVIX BIOPSY	LSIL	3		
21	G972/12	70	F	451127	CERVIX BIOPSY	CDA	3		
22	G255/12	75	F	7142	CERVIX BIOPSY	CIS	3.75	3.78	0.16
23	G1306/11	45	F	41452	CERVIX BIOPSY	CIS	3.75		
24	G1441/11	65	F	30515	CERVIX BIOPSY	CIS	3.8		
25	G1950/11	39	F	64607	CERVIX BIOPSY	CIS	3.75		
26	G2034/11	58	F	455787	CERVIX BIOPSY	CIS	4.05		
27	G2038/11	54	F	446175	CERVIX BIOPSY	CIS	3.55		

28	G36/12	60	F	258710	CERVIX BIOPSY	ISCC-LS -K-WD	4.15		
29	G128/12	60	F	107738	CERVIX BIOPSY	ISCC-LS -K-WD	4.55		
30	G380/12	72	F	108235	CERVIX BIOPSY	ISCC-LS -K-WD	4.15		
31	G399/12	50	F	113552	CERVIX BIOPSY	ISCC-LS -K-WD	4.66	4.55	0.26
32	G431/12	82	F	12940	CERVIX BIOPSY	ISCC-LS -K-WD	4.68		
33	G925/12	45	F	170643	CERVIX BIOPSY	ISCC-LS -K-WD	4.65		
34	G971/12	47	F	206829	CERVIX BIOPSY	ISCC-LS -K-WD	4.88		
35	G1005/12	58	F	235249	CERVIX BIOPSY	ISCC-LS -K-WD	4.66		
36	G1/12	43	F	69525	CERVIX BIOPSY	ISCC-LC-NK-MD	5.21		
37	G9/12	60	F	3148	CERVIX BIOPSY	ISCC-LC -NK-MD	5.32		
38	G22/12	42	F	8946	CERVIX BIOPSY	ISCC-LC -NK-MD	5.32		
39	G38/12	37	F	10761	CERVIX BIOPSY	ISCC-LC -NK-MD	5.19		
40	G64/12	30	F	2199	CERVIX BIOPSY	ISCC-LC -NK-MD	5.19		
41	G67/12	50	F	20557	CERVIX BIOPSY	ISCC-LC -NK-MD	5.21	5.25	0.06
42	G99/12	47	F	29665	CERVIX BIOPSY	ISCC-LC -NK-MD	5.2		
43	G102/12	50	F	29058	CERVIX BIOPSY	ISCC-LC -NK-MD	5.32		
44	G127/12	42	F	3109	CERVIX BIOPSY	ISCC-LC -NK-MD	5.2		
45	G141/12	55	F	1482	CERVIX BIOPSY	ISCC-LC -NK-MD	5.32		
46	G185/12	27	F	11324	CERVIX BIOPSY	ISCC-PD	7.15		
47	G594/12	50	F	21732	CERVIX BIOPSY	ISCC-PD	7.1		
48	G700/12	65	F	8556	CERVIX BIOPSY	ISCC-PD	7.36		
49	G701/12	60	F	208454	CERVIX BIOPSY	ISCC-PD	7.01		
50	G839/12	42	F	34289	CERVIX BIOPSY	ISCC-PD	7.16	7.2	0.11
51	G177/11	65	F	57766	CERVIX BIOPSY	ISCC-PD	7.29		
52	G1844/11	65	F	404874	CERVIX BIOPSY	ISCC-PD	7.16		
53	G1366/11	50	F	44485	CERVIX BIOPSY	ISCC-PD	7.29		
54	G2064/11	55	F	458487	CERVIX BIOPSY	ISCC-PD	7.36		
55	G1541/11	75	F	326715	CERVIX BIOPSY	Adeno CA-MD	6.14		
56	G1603/11	42	F	339147	CERVIX BIOPSY	Adeno CA-MD	6.21		
57	G1644/11	65	F	57746	CERVIX BIOPSY	Adeno CA- MD	6.1	6.13	0.04
58	G98/12	48	F	476511	CERVIX BIOPSY	Adeno CA-WD	6.14		
59	G1259/11	50	F	37314	CERVIX BIOPSY	Adeno Squamous CA	6.06		

60	G1363/11	60	F	272035	CERVIX BIOPSY	Adeno Squamous CA	6.1		
61	G849/11	67	F	35536	CERVIX BIOPSY	Clear cell Adeno CA	6.21		
62	G785/12	45	F	234584	CERVIX BIOPSY	Clear cell Adeno CA	6.14		

Key to Master Chart

Cx	:	Cervix
CRC	:	Chronic Cervicitis
SM	:	Squamous Metaplasia
LSIL	:	Low Grade Intra Epithelial Lesion
CDA	:	Condyloma Acuminatum
ISCC	:	Invasive Squamous Cell Carcinoma
WD	:	Well Differentiated
MD	:	Moderately Differentiated
PD	:	Poorly Differentiated
LC	:	Large Cell
K	:	Keratinising
NK	:	Non – Keratinising
CARCINOMA	:	Carcinoma

ABSTRACT

Study of nucleolar organiser regions (AgNOR) by silver staining technique has been found to be a reliable indicator of cell proliferation and in turn the malignant potential of a lesion.The objective of this study was to evaluate the role of AgNOR in differentiating benign and precancerous lesions from cancerous lesions in cervical biopsy specimens.

This was a prospective study done at Pathology department Coimbatore Medical college Hospital during the period August 2011-July 2012 .AgNORs were counted in biopsies of various lesions of cervix from 62 patients. The mean number of AgNOR per nucleus was significantly higher in cervical intraepithelial neoplasia and carcinoma as compared to chronic cervicitis with squamous metaplasia and normal cervix. This method can be useful as an adjunct to histopathology in diagnosing doubtful cases.

KEY WORDS: AgNOR, Squamous metaplasia, cervical intra epithelial neoplasia, cervical carcinoma